The influence of pelagic life history on the quality of tropical goatfish (family Mullidae) at settlement

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ABSTRACT

High variability in the replenishment of coral reef fish populations by pelagic larvae has been extensively documented. Little is known of the mechanisms underlying this variability or the processes within the plankton that determine the growth and developmental rates of larval fish. Ultimately, these processes will not only influence numerical abundance but also the quality of the recruiting larvae. A common assumption is that fish settle to a reef with similar probabilities of survival and success. The physical condition (e.g. size, biochemical composition and muscle development) of the settling reef fish, and how that interacts with their performance (e.g swimming speed), may influence which individuals survive to join the reproductive population. This study examines how processes within the pelagic life stage influence the quality of newly settling reef fish and explores the ramifications of variable quality to the dynamics of reef fish populations.

The ecology of the pelagic life-stages of goatfish (family Mullidae) was investigated from samples collected in the Lizard Island section of the northern Great Barrier Reef (GBR) (1986 - 1991). A plankton-mesh purse seine was used to collect pelagic stage fish from around small aggregation rafts (1 x 1m). Patterns of distribution around the Lizard Island fringing reef and across the GBR lagoon were examined. Five species were caught, the most abundant being *Upeneus tragula* and *U. moluccensis*. Pelagic stage mullids were found to form large schools (1000+ individuals) that comprised of a number of species (as many as 5), each with a broad size distribution. The largest individuals of each species caught were usually competent to settle.

A distinct metamorphosis accompanied settlement to the reef. Fish changed pigmentation from their silvery pelagic colouration to a mottled cream. Over the same 6 - 12 h period, major restructuring occurred to the barbel sensory system. These are outgrowths of the gustatory system, consisting of batteries of tastebud cells, and are important for the detection and capture of prey items after settlement. Barbel length abruptly increased (up to 52 %) as did mean taste-bud size (up to 100%). Barbels were found to vary markedly in their state of development at settlement.

Age, size and body mass at settlement were examined for goatfish, *Upeneus tragula*, among five stations across the GBR lagoon, over time periods ranging from days to three years. Larval durations and fish length showed equally high levels of variability (ranges: 25 - 37d, 19 - 31 mm standard length respectively). Significant differences in age, length and weight at settlement were found at all spatial and temporal scales. Growth rates averaged over the whole larval period ranged from 0.55 - 1.0 mm/d. Similarly high levels of variability were found in the biochemical composition of newly settled *U. tragula* over ten samples collected over two summers (1990/91, 1991/92). Relationships of nine commonly used measures of fish condition with fish length were very poor. Maximum non-sustainable

swimming speed and biochemical composition were particularly poorly correlated with fish size ($r \leq 0.2$).

Two experiments examined the extent to which food availability and water temperature during the late larval stages influenced the high levels of variability in age and body characteristics of *U. tragula* at settlement. Feeding history influenced size, age, muscle development and body composition of fish at settlement. Water temperature (25 - 30 °C range) significantly influenced only age at settlement. These experiments suggested that the processes of growth and differentiation for late pelagic stage fish dissociated from one another under certain environmental conditions. Over a natural range, water temperature did not dissociate growth and developmental rates. Food availability strongly influenced the relationship between these processes and ultimately governed the age and condition of the fish at metamorphosis. Differences in the temperature regime and feeding history within the pelagic life-stage accounted for much of the variability in growth characteristics among samples.

Two preliminary experiments examined whether the size or body condition at which a fish settled biased the probability of it surviving the initially high predation pressure. Results suggest that predation by the lizardfish, *Synodus variegatus*, was random with respect to both size and biochemical composition of the newly settled goatfish, *U. tragula*. This suggests that the influence of the pelagic life history will extend well into the post-settlement stages and may ultimately influence which individuals join the reproductive population.

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Chapter 1

GENERAL INTRODUCTION

1.1 BACKGROUND

Variable replenishment is a feature of all coral reef fish populations (reviews by Doherty & Williams 1988, Doherty 1991). Recently, some success has been achieved in linking together the numerical abundance of the disparate life stages of reef fish (egg, recruit, adult stages). Meekan *et al.* (In press) found significant correlations between the timing of spawning in the damselfish, *Pomacentrus amboinensis*, and the temporal patterns of larval supply and recruitment. A similar relationship was found for the Caribbean damselfish, *Stegastes partitus* (Robertson *et al.* 1988). Milicich *et al.* (In press) found that the availability of larval fish in the vicinity of a reef correlated with weekly patterns of recruitment to that reef. Furthermore, Doherty and Fowler (In press) found that interannual variation in recruitment strength can be preserved in the age structures of the adult population over decades at a reef-wide scale. These studies have shown that much of the variability in abundance patterns of particular life stages can be correlated with the numerical abundance of the earlier life stages. However, there is a large amount of variability unexplained by these relationships, especially at the within reef scale.

Much of the variation in recruit abundance has been attributed to processes within the planktonic phase of the life cycle (e.g. Richards & Lindeman 1987, Doherty 1991). This is important as relatively small changes in the demographic parameters during the early life stages can result in dramatic fluctuations in recruitment (May 1974, Houde 1987, 1990, Miller *et al.* 1988, Beyer 1989). Compensatory mechanisms (Fogarty *et al.* 1991), such as density-dependent growth and mortality rates, may regulate recruitment fluctuations (Jones 1973, Jones & Henderson 1988, Beyer 1989, Beverton & Iles 1992). Changes in growth rate may have additional effects on size-at-age relationships, developmental rates and body composition which in turn influence future probabilities of mortality and reproduction.

Although considerable effort has been directed toward the numerical analysis of recruitment events and their consequences, relatively little is known about the more subtle effects of changes in growth rate and body composition. Indeed neither the mechanisms that drive differences in growth

rate and body condition nor their demographic consequences have been adequately examined in recruiting fish. This is especially true for coral reef species. The extent to which variation in body condition at settlement biases probabilities of future survival or how such effects might flow on to adult growth and reproductive rates is unknown.

Not only are the consequences of variation in body condition poorly understood but the mechanisms underlying such variation have seldom been examined. Recent studies have shown that parental condition can influence the viability, growth and development rates of larvae (e.g. Thorpe *et al.* 1984, Schreck *et al.* 1991, Kamler 1992). Feeding and environmental regimes during the pelagic phase may interact with the legacy of parental condition to further modify the condition or quality of newly settled fishes. In this context the status of the parent and pelagic phase of the life history are responsible for more than the numerical input of larvae. Qualitative differences in newly settled fish both within and among cohorts may be a powerful influence on demographic performance. Ultimately both quantitative and qualitative information will be required to understand fluctuations in the abundance of recruits and their consequences.

The present study addresses the topic of variation in condition and quality of newly settled reef fishes. The primary goal is to understand the mechanisms underlying this variability, especially as a consequence of events during the pelagic phase of the life history. The demographic and life history focus is the late pelagic phase and the process of transition from pelagic to demersal existence. Specifically the study describes the ecology of the pelagic stages of a particular group of reef fish, and the changes that occur at the metamorphosis associated with settlement. This information is used to interpret patterns of spatial and temporal variability demonstrated to exist in the age, size, and body composition at settlement. Experiments examine the extent to which food availability within the pelagic and water temperature drive the levels of variability observed. Lastly, the study examines whether the levels of variability found in the size and body condition at settlement influence which individuals survive the initially high predation pressure on the reef. By combining ecology with physiology and biochemistry this study provides an integrated perspective of the dynamics and importance of settlement not previously obtained.

Members of the family Mullidae, or the goatfishes, were targeted for this study. These make a good model for tropical reef fish population

dynamics, and an interesting comparison to the nest building territorial pomacentrids on which most recruitment studies have been conducted. As a group they are pelagic spawners, with a solitary or schooling habit and with members that occur in all reef habitats. Mullids are a common component of the ichthyoplankton and are in highest abundance in the upper 6m of the water column (Leis 1991a). They are one of the few groups in which pelagic stages of a wide size range can be caught in very high numbers (1000+), identified to species (for the most common species) and maintained in tanks with minimal mortality. This enables experiments using wild caught fish that are mid-way through their pelagic phase. This reduces rearing artefacts and maintains natural levels of genetic variability, thereby increasing the applicability of tank experiments to the field situation. Typical of most reef fish, mullids undergo a change in pigmentation and behaviour associated with settlement. Mullids are unique in having a pair of barbels on the chin that represent outgrowths of the taste system. These are important in the detection and capture of prey after settlement. The discrete and easily accessible nature of these barbels, and their importance to foraging once settled, makes them perfect for the study of the factors that influence sensory system development in reef fish. Together these attributes make mullids ideal for a detailed study of the pelagic-demersal transition.

An understanding of the extent and nature of the rapid changes that have become synonymous with settlement is crucial to enable identification of factors that will influence the abundance patterns and survival of newly settled reef fish. Metamorphosis involves a major change in virtually all facets of the fishes' ecology. For instance, a change in the sensory cues received by the fish (e.g. olfactory and visual), changes in food types and availability, the modification of foraging techniques used in the pelagic to prey types never before encountered, a change in the type and intensity of intra- and inter-specific interaction, amongst many other. This metamorphosis has been well documented for temperate flatfish and bonefish, where dramatic changes in morphology and physiology have been noted (e.g. Ryland 1966, Pfeiler & Luna 1984, Markle *et al.* 1992). In contrast, for reef fish only cursory references have been made to changes in pigmentation (Caldwell 1962, Wallace 1967) and morphology (Leis & Rennis 1983). By examining the changes that occur in the gustatory system through pelagic development and settlement the current study identifies settlement as more than a change in pigmentation, but rather a crucial developmental transition required for the appropriate functioning of the settled fish.

The present study re-evaluates the evidence for the mechanisms underlying settlement in reef fishes. The ability of reef fish to delay metamorphosis and settlement has been stated as the reason for much of the variability found in the age and size at settlement (e.g. Victor 1986b, Cowen 1991, Wellington & Victor 1992). Fish that do not come across an appropriate settlement cue, or are swept away from the reef environment by offshore currents (Victor 1987) are said to delay settlement until a suitable habitat is found. Alternatively, metamorphosis and settlement may be simply the next step in a developmental continuum whose rate can be altered by environmental (e.g. temperature) and biotic factors (e.g. nutrition) within the pelagic, but which leaves little room for choice in the timing of settlement. One of the difficulties in addressing these hypotheses has been the paucity of data on the levels of variability in the ages and sizes at settlement over a range of spatial and temporal scales. The present study provides such information and examines evidence for the competing hypotheses. Furthermore, it suggests the processes that determine the growth and mortality rates within the planktonic stages and the scales on which they operate.

This is the first study of a coral reef fish population to examine the levels of variability in the body condition or quality of fish at a key developmental stage, such as settlement. For larval fish it has been suggested that size is strongly related to survival through a positive feedback loop, involving higher swimming speeds, prey capture success and growth rates (Miller *et al.* 1988, 1992, Bailey & Houde 1989). It is important to our understanding of reef replenishment to know whether these size based advantages hold for newly settled reef fish, and whether potential success can be easily predicted in the field. By examining the inter-relationships between size and commonly measured aspects of body condition and survival potential (e.g. burst speed), this study stresses the complex and multi-dimensional nature of fish condition.

1.2 THESIS OUTLINE

The rationale used in the organisation of this thesis is the belief that separate sections should be complete contributions in themselves, although linked by a common theme. To simplify synthesis of the information contained within each section, summaries are included at the start of each data chapter. Eight chapter comprise this dissertation. Six of these chapters (2 - 7) represent submitted papers, one of which is published (Appendix 1).

Chapter 2: *Late pelagic-stage goatfishes: distribution patterns and inferences on schooling behaviour*

The spatial and temporal abundance patterns of pelagic-stage goatfish across the northern Great Barrier Reef are examined from 1986 to 1991. Purse-seines of fish around aggregation rafts are used to sample schools of goatfish, and compared to catches from automated light traps. The species and size composition of schools is examined and inferences are made on schooling dynamics and their relevance to the potential magnitude of larval dispersal. The most abundant goatfish caught, *Upeneus tragula*, that is the subject of subsequent chapters is introduced.

Chapter 3: *Development and changes at settlement in the barbel structure of the reef fish,* Upeneus tragula

What metamorphosis actually means for the sensory system of a goatfish is examined in detail. The barbel system is an extension of the gustatory and olfactory system, that is only used in the detection and capture of prey items once settled. Development is recorded morphometrically and histologically from their initiation in the mid larval stage through to the settled juvenile. Changes that occur at settlement and factors that control their development are compared to other aspects of the teleost sensory system.

Chapter 4: *Variability in age and size at settlement: how much is there and what can it tell us about the process of settlement?*

Variability in the size and age at settlement is examined for *Upeneus tragula* among five sampling stations across the northern Great Barrier Reef, over time periods ranging from days to three years. The use of sagittal otoliths for daily ageing is validated. The current view of the mechanism underlying variability in settlement is re-evaluated.

Chapter 5: *Quality of the reef fish,* Upeneus tragula, *at settlement*

The quality of newly settled goatfishes is examined over ten samples from one location off Lizard Island, on the northern Great Barrier Reef. Nine commonly used methods of assessing fish condition are used and compared. Fish size is compared to biochemical composition, growth rates and maximum swimming speed of individual fishes. The applicability of size-based theories of quality to newly settled fish is discussed.

Chapter 6: *Effects of feeding history during the pelagic phase on the growth characteristics of the reef fish,* Upeneus tragula, *at settlement*

The influence of feeding history within the larval stage on the growth attributes of *Upeneus tragula* at settlement is investigated in detail. Size, muscle development, age, body constituents and feeding rates are examined. The importance of adaptability to variable larval feeding regimes is discussed.

Chapter 7: *Effect of temperature during the pelagic life stage on the growth characteristics of the reef fish ,* Upeneus tragula, *at settlement*

The influence of temperature of the water column during the larval stage on the development and differentiation of *Upeneus tragula* to settlement is examined. The magnitude of the effects found are compared to the feeding experiment and their relative importance in governing the relationship between somatic growth and differentiation is discussed.

Chapter 8: *General Discussion*

The major findings of the study are synthesised and the influence of predation on the size structure and quality of newly settled fish is discussed.

1.3 TERMINOLOGY

Due to the plethora of terms used to label the diverse life stages of fish, clarification of the terms used in this study is necessary. Definitions of terminology follow Blaxter (1988). The term "embryo" refers to developmental stages to hatching; "larva" refers to stages of development from hatching to metamorphosis (synonymous to “pre-settlement”, Kingsford 1988) and "juvenile" from metamorphosis to first spawning (synonymous with “post-settlement”). The term “pelagic stage” refers to the late larval stage that have formed active schools. “Settlement” is used to designate the period of transition from open water to benthic habitat. This may be abrupt or gradual (Kaufman *et al.* 1992). “Recruitment” corresponds to post-settlement survivorship until initial census observation (Richards & Lindeman 1987).

Chapter 2

Late pelagic-stage goatfishes: distribution patterns and inferences on schooling behaviour

2.1 Synopsis

Spatial and temporal abundance patterns of pelagic-stage goatfish (Family Mullidae) across the northern Great Barrier Reef were examined (1986 to 1991). From these data inferences were made on the behaviour and schooling dynamics of this largely unstudied life-stage. Sampling was conducted using both a plankton-mesh purse-seine deployed around small aggregation rafts (1 x 1 m) and automated light traps. A comparison of the two techniques showed no difference in the size composition of fish caught. Neither the total number of mullids caught at a sampling station nor their minimum size was related to the current speed as measured by drogues (0 and 5m depth). A comparison of the species composition of raft and light trap samples with that of fish settling onto the reef below the rafts showed that, of the 11 species known to occur in these waters, only five were caught by the two devices. Size frequencies of catches around rafts suggested that at times multiple schools were present within a 90m sampling area (i.e. one site). Schools were comprised of a number of mullid species, each having a broad size range [e.g. *Upeneus moluccensis*, 20 to 56 mm SL]. The largest individuals were competent to settle. Samples from light traps deployed off the reef around Lizard Island for 280 days over three summers (1986/87 to 1988/89) found that late pelagic-stage mullids occurred close to the reef in irregular pulses. Raft sampling at the backreef found a similar pattern. Pelagic mullids collected in purse-seine samples were consistently common in inter-reefal waters. A cross-shelf sampling programme involving six stations stretching from the inshore Turtle Group, past Lizard Island to 3 km off Carter Reef was conducted. Catch composition and abundance was found to vary among locations over three monthly samples. *Upeneus tragula* and *U. moluccensis* dominated the catch at all but the Outer Barrier station, where the genus *Parupeneus* was most abundant. Findings stress the complex interaction between behaviour, ontogeny and abundance patterns, and the potential for late pelagic-stage fish to influence their recruitment patterns.

2.2 INTRODUCTION

Most reef fish have a pelagic larval phase that disperses an unknown distance from parental reefs. Initially, larvae are small, naive and have limited mobility, but by the end of the pelagic phase the fish of many taxa are well-developed, highly mobile and sensorily competent. At this stage individuals may exert considerable control over their distribution and final destination, and the consequences of their behaviour, physiology and mobility may extend well past settlement and into the benthic reef population itself. Indeed, recent studies have shown or implied that factors which influence the late-pelagic life-history stages may regulate numbers recruiting to the adult population (Houde 1987, 1989a, Miller *et al.* 1988, Davis *et al.* 1991, Doherty 1991, Milicich *et al.* In press).

Despite their inferred importance, the late-pelagic stages have generally escaped emphasis in ecological studies, almost certainly due to the difficulties of capturing them with conventional ichthyoplankton techniques (Brander & Thompson 1989, Choat *et al.* In press). Light traps and the use of a plankton-mesh purse seine around drifting objects are two recently developed techniques that alleviate many of the sampling problems (Kingsford & Choat 1985, Doherty 1987, Milicich 1988). By exploiting certain behavioural attributes of the fish, these techniques are capable of concentrating and collecting sufficient numbers to allow meaningful descriptions and comparisons to be made across a range of spatial and temporal scales. In addition, fish are live upon capture and can be used in controlled experiments (e.g. McCormick & Molony 1992), or reared to stages that can be identified to the species level.

These technological developments allow the ecology of the late-pelagic developmental stages of a variety of species to be examined in a detail and with a rigour not previously possible. This study targets a family of reef fishes, the goatfishes (Mullidae), that are characterised by an often lengthy and variable pelagic life history phase. Little is known of any aspect of the pelagic life history stage of this group and this study aims to provide some of the first data on the distribution, abundance and behaviour of these fish. Detailed analyses of these patterns at a range of spatial and temporal scales allows me to generate hypotheses about the behaviour and movement of these fish and speculate how these may influence the recruitment process.

I examine the spatial and temporal distribution patterns of pelagic-stage goatfishes in the vicinity of Lizard Island on the northern Great Barrier Reef. Catches from around aggregation rafts and in light traps are compared to determine which facets of the population each technique targets. Data from automated light traps (Milicich 1992) is re-examined and used in combination with data from aggregation rafts to examine daily patterns of abundance around the Lizard Island fringing reef over three summers, from 1986/87 to 1988/89. Aggregation rafts deployed at six stations spanning the northern continental shelf are used to examine large scale spatial abundance patterns and species composition. By sampling on a wide range of spatial and temporal scales with a number of techniques this study provides a unique insight into a life stage not previously examined in detail. Evidence presented here reveals a complex school organisation, with species-specific behaviours and distribution patterns.

2.3 MATERIALS AND METHODS

2.3.1 Sampling gear and species identification

Raft design

One-metre square aggregation rafts were made from PVC tubing, with cross-struts for strength. A ring was attached to the cross-struts mid-centre of the raft for mooring attachment. The framework was covered with thick green plastic. As a three dimensional floating structure aggregates fish better than a two-dimensional one (Hunter & Mitchell 1968) flaps of plastic (15 cm down) were attached to the undersurface of the raft together with 1 m long (10 cm wide) weighted streamers. These rafts were used for all but the Outer Barrier sampling.

Sampling on the Outer Barrier Reef used 1 x 1 m aggregation rafts with a wooden framework (3 x 4 cm) to which a plastic mesh and a layer of coconut fronds were attached. Two weighted frond tips 1 m in length were secured to the middle of the undersurface of the raft. Outer Barrier samples were compliments of an unpublished study of raft associated fauna (J. H. Choat & B. A. Kerrigan, JCU).

In both instances, the ichthyoplankton aggregated under the rafts were sampled with a plankton-mesh purse seine (14 x 2 m, 0.5 mm mesh) (see Kingsford & Choat, 1985, for design).

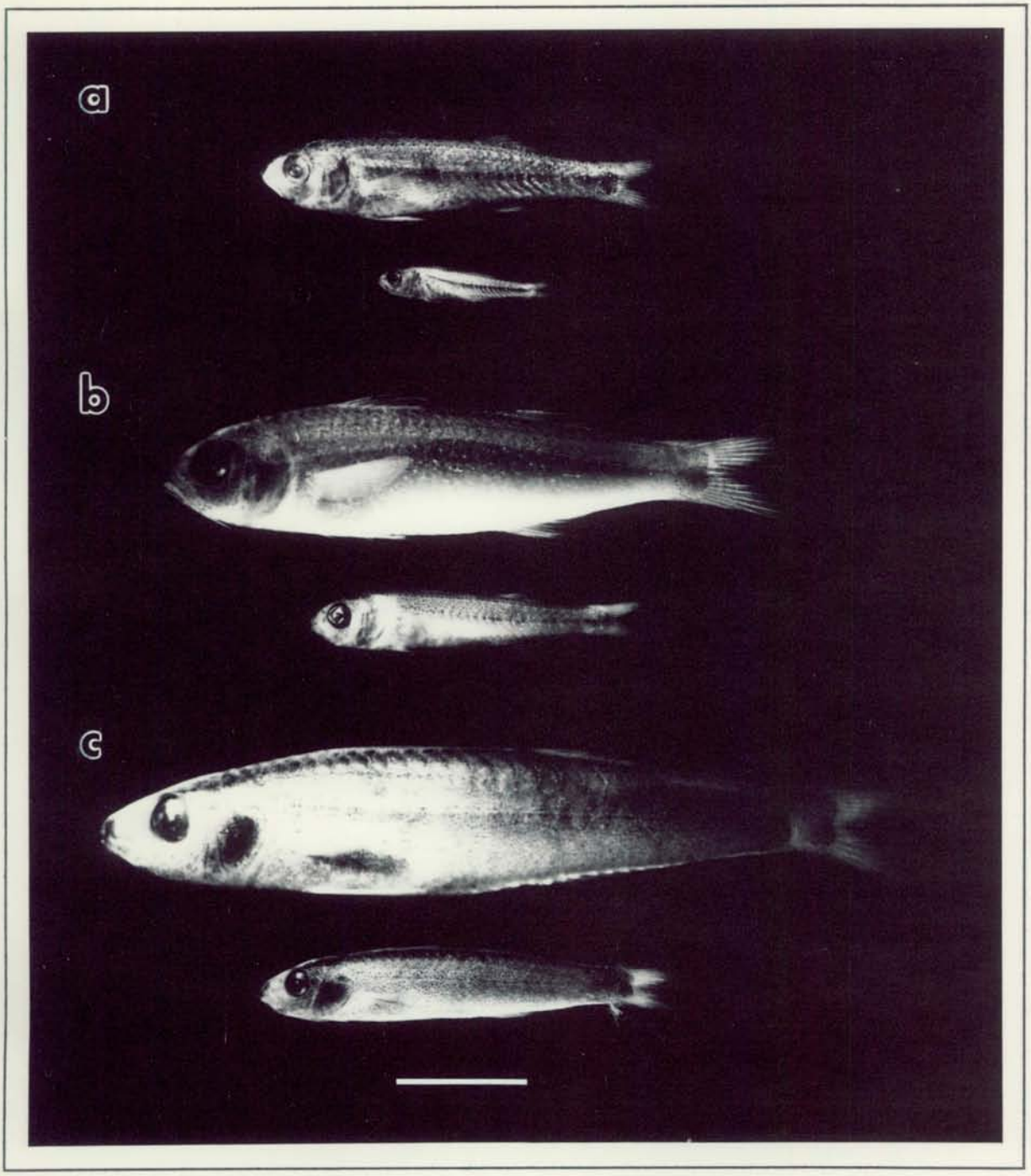

Figure 2.1. Approximate size range of the three most abundant species of pelagic-stage mullids caught under aggregation rafts on the northern GBR, in order of overall abundance. (a) *Upeneus tragula*; (b) *Upeneus moluccensis*; (c) *Parupeneus heptacanthus* (scale: 10 mm).

Light trap design

Design and construction of the light traps follow that of Doherty (1987).

Species identification

Species of pelagic mullid were identified by bringing them back to the Lizard Island Research facility and rearing them in tanks on *Artemia* sp. until they settled and could be identified by adult colouration and body shape. For the two most common species (*Upeneus tragula*, *U. moluccensis*) sufficient were caught and reared to allow the progression of size and form to be described throughout the pelagic colour phase (from approx. 7.5 mm SL through to settlement). Figure 2.1 shows the approximate size range of pelagic-stage individuals of the three main species examined in this study. The actual largest and smallest individuals from the samples were not fit for photography.

2.3.2 Methodological comparison

To enable the comparison of catches from purse-seine samples around rafts during the day with light-traps samples at night, the size distribution of catches around Lizard Island were compared using data from the 1989/90 sampling season. Data used were from the three nights when mullids were in highest numbers in the light traps.

2.3.3 Species selectivity

The species selectivity of the aggregation devices was examined by comparing the species-composition of the raft (from Dec 1988 to March 1989) and light trap samples (during Oct 1989 to Jan 1990) with fish censuses of the reef directly under the rafts, conducted on a daily basis during 1988/89 and haphazardly through the 1989/90 sampling period. Two permanent 50 x 10 m census strips were established under the northern and mid rafts at the Granite and Turtle sites (Fig. 2.2). Census strips were laid parallel to the shore, one on the shallow ledge (~ 3 m depth) which abutted the wall, the other on the reef slope extending to the bottom of the reef at 13 - 23 m. Newly settled mullids were identified to species.

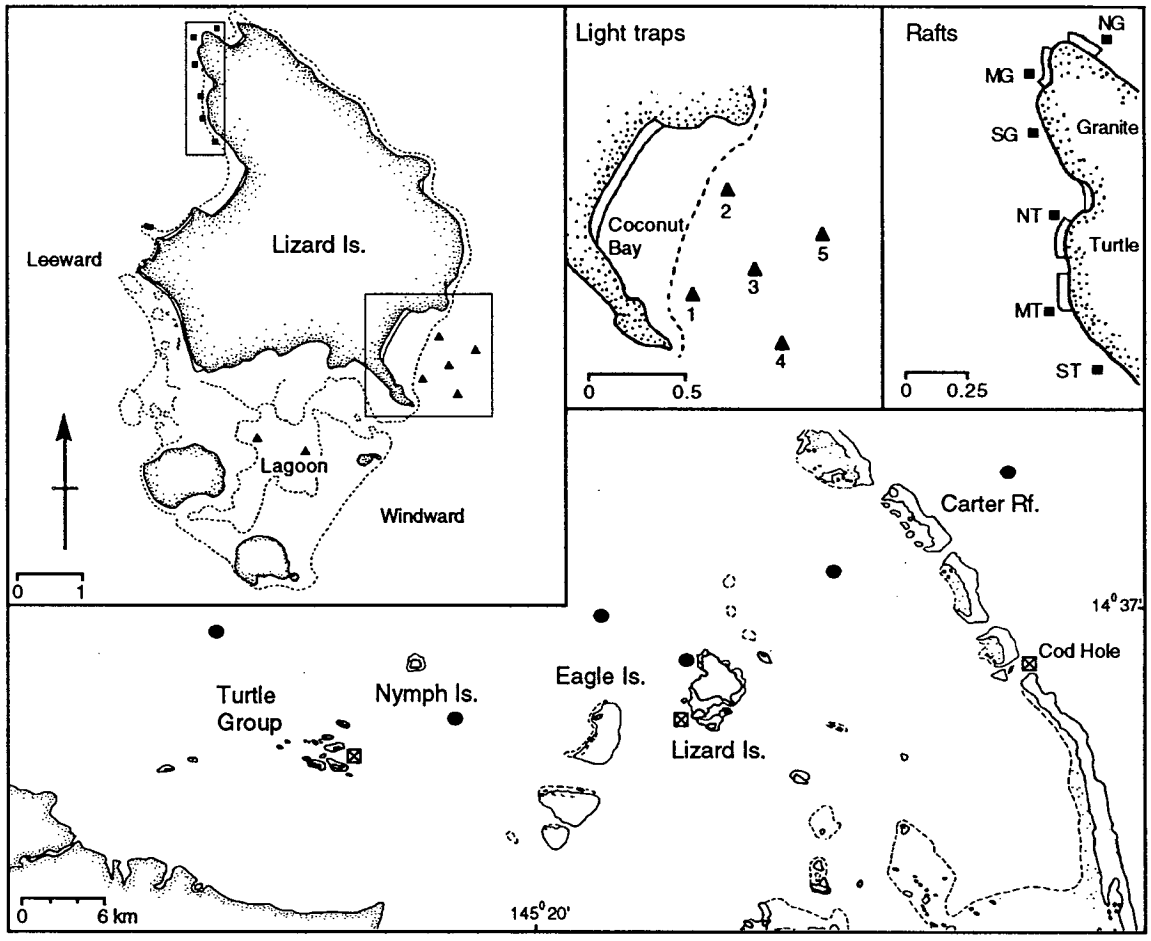

Figure 2.2. Stations across the northern Great Barrier Reef where mullid samples were collected using aggregation rafts (large solid circles). At each station two sites of four rafts were sampled. The map of Lizard Island shows the position of rafts (squares) and light traps (triangles) for the temporal sampling programme. Details of the intensive sampling surveys with light traps at the reef front (1986/87), and rafts at the backreef (1988/89) are given (inset). Crossed-square symbols represent stations at which dataloggers were placed at 10 - 12m depth to record water temperature (used in Chapter 7).

2.3.4 Sampling design

Spatial and temporal patterns at Lizard Island

From the 3 months between Dec. 1988 to March 1989, 2 to 3 permanent raft moorings (200 m apart) were located on the reef edge at each of two sites (Granite and Turtle) on the back-reef of Lizard Island (Fig. 2.2) in 10 - 23 m water depth. Plastic rafts were clipped to the moorings and the ichthyofauna aggregated was sampled daily between 07.00 and 10.00 hrs. Exceptions were when strong N-W winds (> 20 knots) and rough conditions made seining unsafe. At these times rafts were visually censused and removed for a period to allow fish to disperse before rafts were re-deployed.

The sampling protocol of the fauna aggregated by both raft types was similar. Initially, a visual census of the fauna under the raft was done by a snorkeller after which the raft was unclipped from the mooring. Once the raft had drifted sufficiently far from the mooring to allow an unobstructed path, the aggregated fauna was then sampled using a 14 x 2 m plankton-mesh purse seine (0.5 mm mesh) (Kingsford & Choat 1985). A comparison of censused versus seined data found that the unclipping procedure had little effect on the ichthyofauna caught.

To augment this raft sampling programme, data from an unpublished study of pre-settlement reef fish around Lizard Island (Milicich 1992) was re-examined. This study overlapped temporally with the current study (summers 1986/87 to 1988/89).

Automated light traps (Doherty 1987) were moored at the surface off Lizard Island's fringing reef and sampled on a daily basis. Trapping methodology is addressed in Milicich (1988) and Milicich *et al.* (In press), so will only be addressed briefly here. The fluorescent light tubes within the traps were on for 3 one-hour periods per night (2100 - 2200h, 2400-0100h, 0300 - 0400h). This staggering of sampling periods was introduced to integrate any effect that time of night or tidal state had on catch rates. During the 1986 - 87 sampling period all traps were positioned seaward of the reef front. Four of the five traps were placed in a square, with the fifth trap initially placed in the centre (Fig. 2.2). The inner-most traps were about 250 m from the reef edge. After the end of November 1986, the fifth trap was re-deployed further inshore of the other traps at a distance of about 150 m from the reef (Fig. 2.2). This spatial design meant it was possible to look for

consistent differences in the abundance of pre-settlement mullids at particular sites.

During the other 3 years a varying number of traps were moored around the island as the number of habitats sampled increased. Although potentially this allowed inter-habitat comparisons to be made, collection rates in the relevant years were low making such comparisons meaningless. However, as sampling off the reef front was continuous over three consecutive summers it was possible to produce a single time series that was 280 days long (concatenated on a lunar time scale, see Milicich 1992). This allowed Milicich to look for periodicities in the presence of pre-settlement mullids in the waters around Lizard Island. This was achieved using spectral analysis (Jenkins & Watt 1968, Chatfield 1979). Data were logged prior to analysis and subjected to a high-pass filter (Milicich 1992). Spectral analysis breaks down the variance of a time series, apportioning it to sinusoidal curves of varying frequency so that any significant cycles that are present in the data can be identified. Autocorrelation analysis was then used to further pinpoint any significant periodicities that were identified (Robertson *et al.* 1988).

Spatial and temporal patterns across the shelf

Five stations across the northern Great Barrier Reef lagoon were sampled once per month for 3 months between Nov. 1989 to Jan. 1990. Station locations were: 9 km N-W of the Turtle Group; 4 km south of Nymph Island; 5 km north of Eagle Island; Lizard Island backreef; midway between Lizard Island and Carter Reef (abbreviated MLC) (Fig. 2.2). At each station two sites were established; one 500 m each side of a stationary mother boat. At each site, 4 rafts were tethered, with 30 m lines linking the rafts to one-another and to a single temporary mooring. Rafts were left for 4 h and then sampled with the purse-seine. Estimates of current speed and direction were made twice over four hours by releasing 2 tethered drogues (0 and 5 m depth) from the back of the mother boat and timing how long it took them to travel 70 m. The bearing was obtained using a hand compass.

Outer Barrier sampling

During Nov 1987 coconut-frond rafts were floated 1.5 to 3 km off Carter Reef. Rafts were tethered together by 30 m lines and were released off

Carter Reef early in the mornings and allowed to drift with the prevailing southerly current along the Outer Barrier Reefs for 4 h before sampling.

2.3.5 School structure

The cross-shelf sampling protocol at each of two sites involved 4 rafts linked by 30 m ropes and attached to a single temporary mooring. The proportion of the total catch for each site associated with each of the four consecutively sampled rafts was examined to give an insight into the size of the schools of the pelagic fish, the integrity of schools and their composition.

This information was also used to examine whether individual rafts in the series could be treated as independent replicate samples of the same population. As there was an auto-correlative trend in the data I could not use conventional parametric statistics to explore patterns in the cross-shelf study. Results are therefore presented graphically.

The deployment of the light traps off the reef-front of Lizard Island during the first year of sampling allowed us to examine the variability in catch rates among replicate, adjacent traps during any single night. Variance-mean ratio (VMR) were calculated across these traps over a range of nights. A high VMR is indicative of a highly clumped distribution that can be most parsimoniously explained by the presence of schooling behaviour. This ratio was contrasted with that determined from the rafts deployed at the back-reef of Lizard Island. It is assumed that there is no behavioural difference between pelagic fish in the front and back of the reef.

The relationship between current speed and the total numbers of fish caught under the moored rafts was examined for two reasons: a) to examine whether current speed in which the rafts were moored would bias the catches of mullids under those rafts (both size and number of fish); b) to make inferences on the ability of mullids to maintain their position under a range of current regimes in the field. Current speed and direction were recorded as part of the cross-shelf sampling programme.

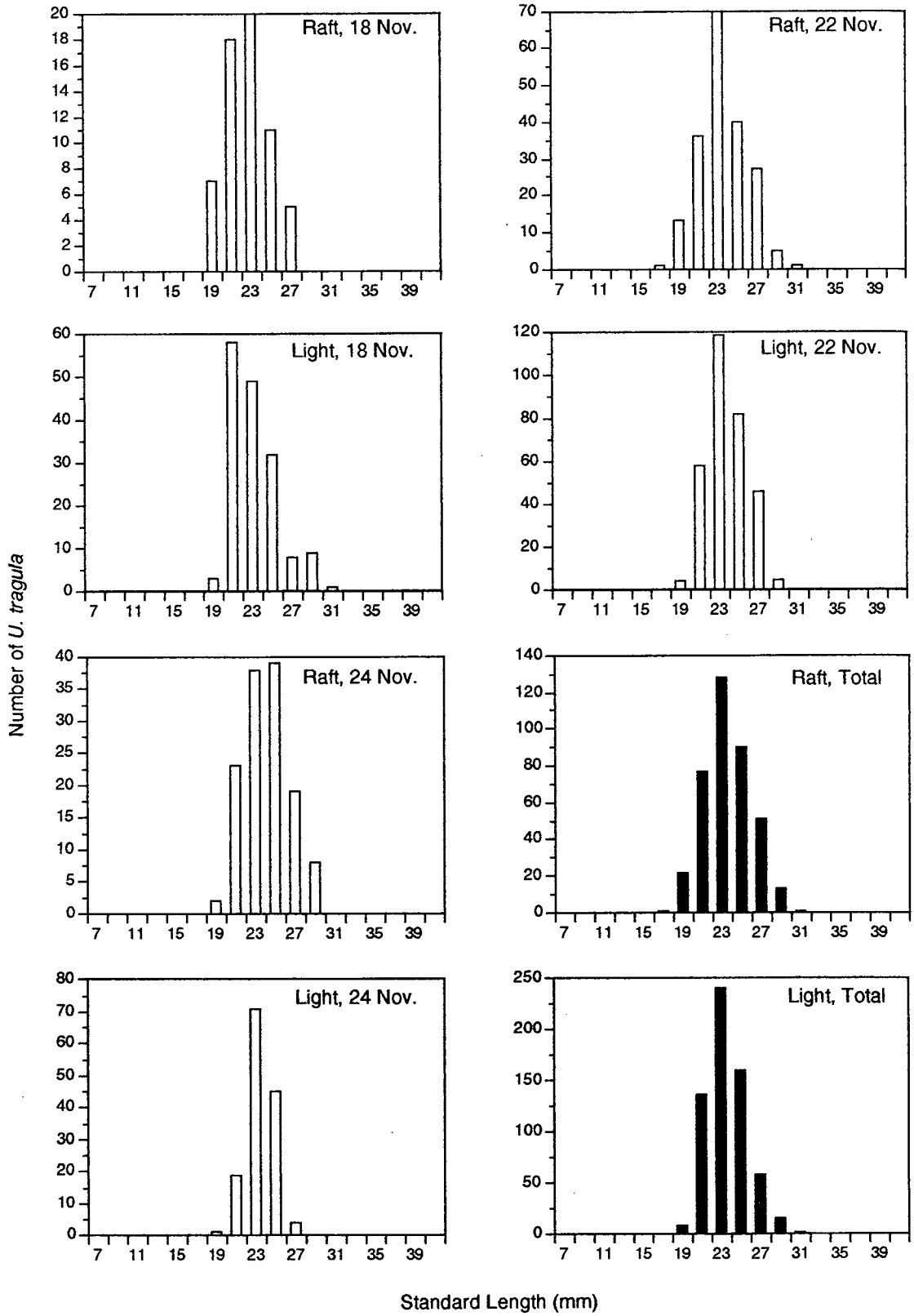

Figure 2.3. Size frequencies of *Upeneus tragula* caught under rafts during the day and light traps at night pooled over all rafts ($n = 4$) and traps ($n = 5, 4, 5$ respectively) for 3 sampling dates. Pooled size distributions over the three sampling dates are also given. Sizeclass mid-points are displayed.

2.4 RESULTS

2.4.1 Methodological comparison

The size distributions of raft and light trap samples were compared to determine whether both methods were sampling the same components of the mullid population. There were minor differences in size distributions on particular days (Fig. 2.3), but these may be due as much to differences in the spatial deployment of the light traps and rafts as to any real bias in the methodology. Light-traps were positioned near the reef-front (2 traps), back-reef (2 traps) and lagoon (1 trap), while rafts were moored only on the back-reef (4 rafts). When the size distributions were summed over the three sampling dates no difference was apparent between the two techniques ($\chi^2_{0.05,5} = 7.958$, $p > 0.170$, Fig. 2.3).

Table 2.1. Species selectivity of aggregation rafts and light-traps for pelagic-stage mullids. Newly-settled stages indicate those species that are potentially available to be caught in the pelagic stage. Ranked abundances of settled-stages on the reef and pelagic-stages under rafts and in light-traps are given (1 most abundant, 10 least). Genera are *Parupeneus*, *Mulloidichthys* and *Upeneus*.

	Rank abundance	
Species	Newly settled to reef	Raft & light-trap
P. multifasciatus	1	-
P. barberinus	2	4
P. cyclostomus	3	-
P. ciliatus	4	5
P. bifasciatus	5	-
M. flavolineatus	6	-
P. indicus	7	-
P. heptacanthus	8	3
P. pleurostigma	9	-
U. tragula	10	1
U. moluccensis	-	2

2.4.2 Species selectivity

Aggregation rafts and light-traps did not sample all the species of pelagic-stage mullids present in the water column at the time of sampling (Table

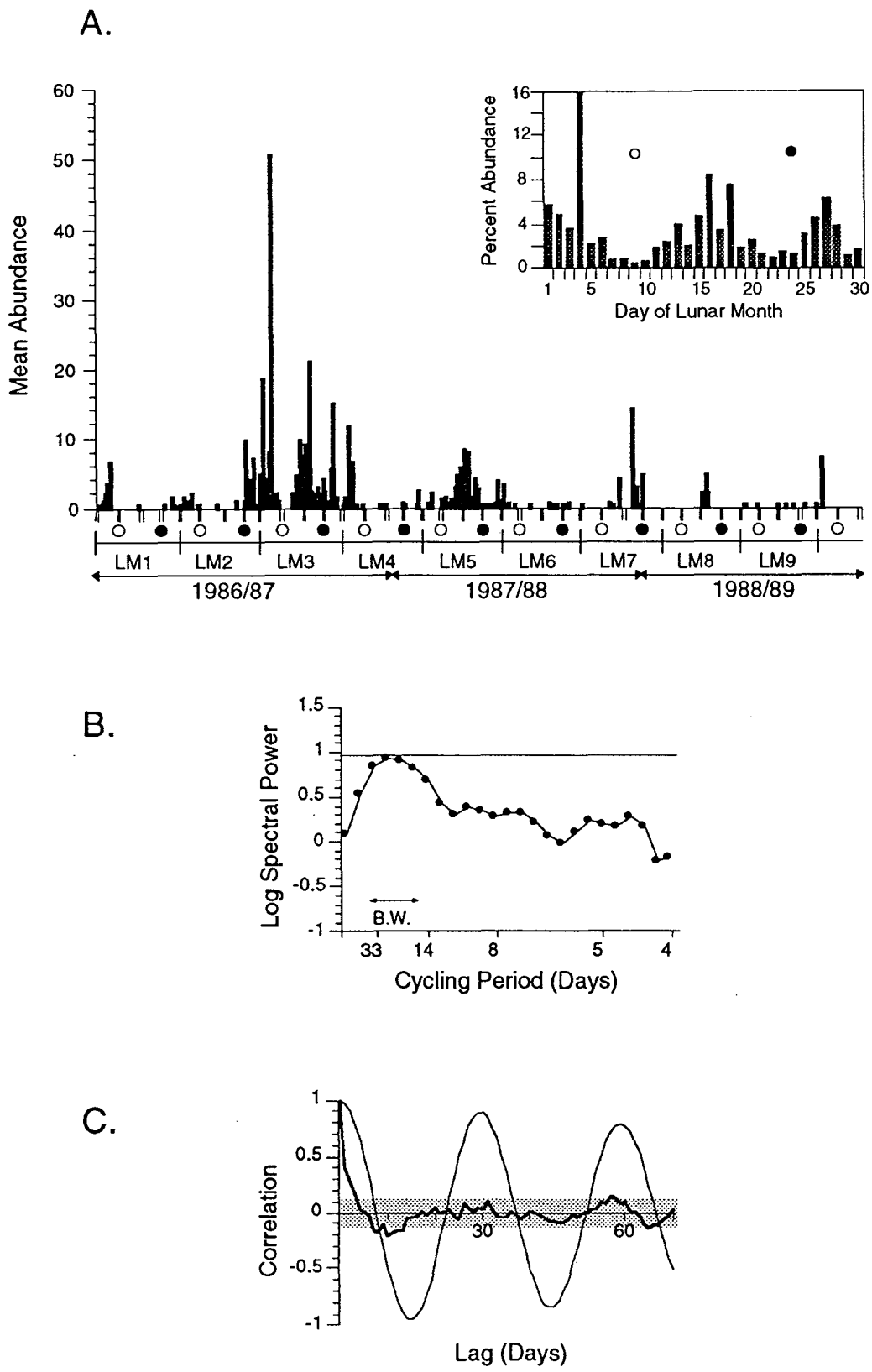

Figure 2.4. Temporal abundance patterns of *Upeneus tragula* on the Lizard Island reef front (modified and reprinted with permission from Milicich 1992). (A) Mean abundance per day for mullids caught in light traps over three sampling seasons (concatenated by lunar month, LM). Inset shows the percent contribution of total abundance from each day of the lunar cycle. (B) The relative importance of the length of a cycle in explaining the abundance patterns in the data, as expressed by spectral power. (C) Comparison of the autocorrelation function of the fish abundance (bold) and a lunar cycle (plain).

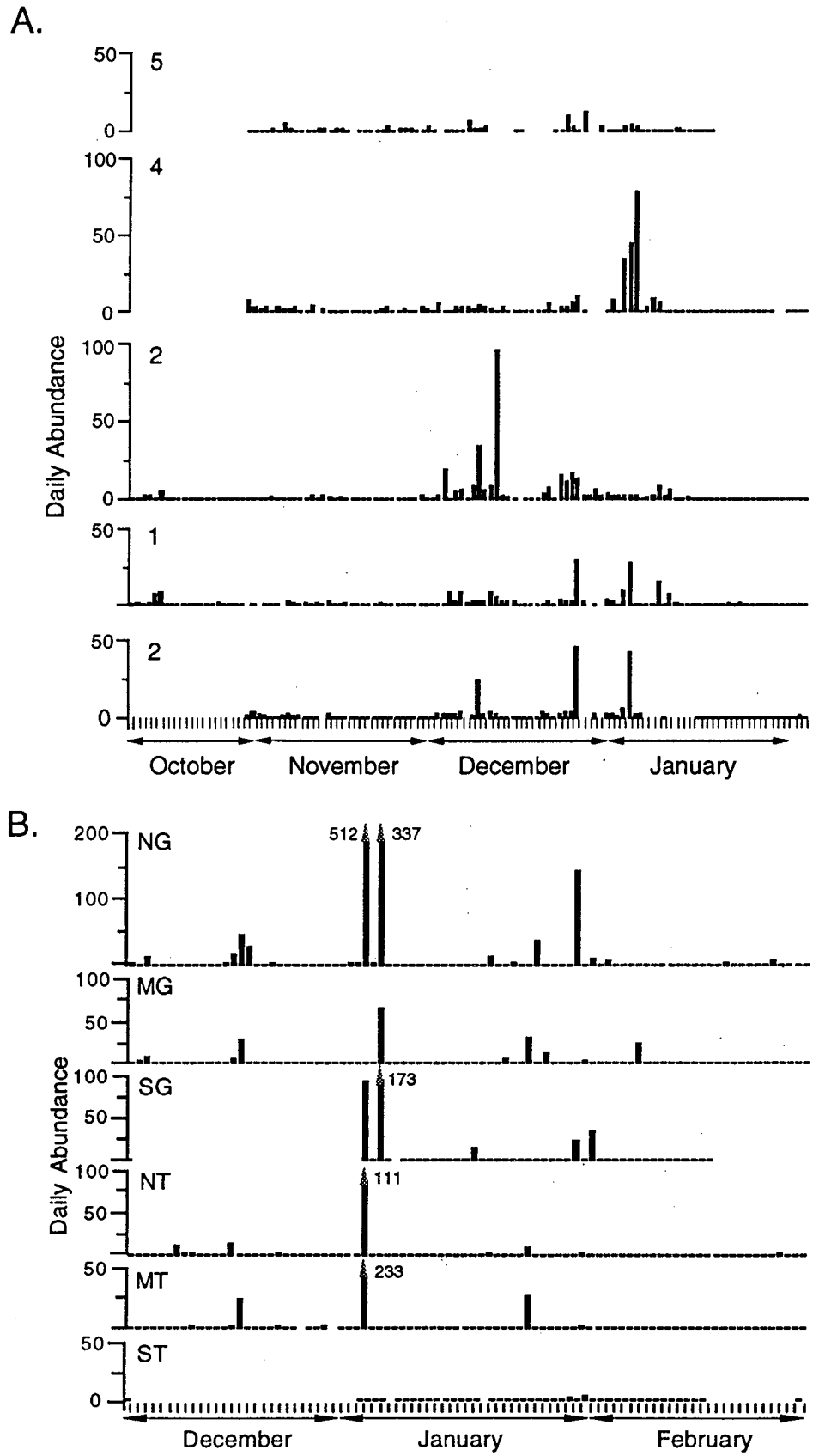

Figure 2.5. Daily catches of *Upeneus tragula* for individual light traps and rafts. (a) Light traps (1 to 5) during the 1986/87 season (reprinted from Milicich 1992). (b) Aggregation rafts (NG - ST) during 1988/89 season. Light trap and raft labels follow those of Fig. 2.2.

2.1). Newly settled fish were used as a measure of what species were present in the water column and potentially available for capture. Of the 10 species which were found to settle onto the Lizard Island backreef during Dec 1988 to March 1989, only 5 occurred under the rafts and within the light-traps (Table 2.1). Furthermore, there was a negative relationship between the abundance of particular species under the rafts and in light-traps and the abundance as newly settled fish on the reef (Table 2.1). Those species which were least associated with the hard reef-substratum dominated the raft and light trap samples. The commonest species, *Upeneus tragula* and *U. moluccensis,* are principally inter-reefal as adults.

2.4.3 Spatial and temporal abundance around Lizard Island

The abundance pattern of *Upeneus tragula* near the reef front is characterised by periods of absence or very low numbers interspersed with short periods of high catches (Fig. 2.4a). Although the spectral analysis identified the presence of a broad peak centred around a 25 day cycle (Fig. 2.4b), the peak was broad and the 25 day periodicity only explained 9% of the variation in *U. tragula* abundance. The autocorrelation analysis confirms this minimal cycling effect, particularly noticeable when compared to the pattern produced by the simulated lunar cycle (Fig. 2.4c).

When five light-traps were moored off the reef-front, all caught similar densities of mullids with the exception of trap 5 which lacked large peaks in abundance (Fig. 2.5a). There was little consistency in the timing of abundance peaks among the traps within Coconut Bay (Fig. 2.5a). However, when mullids were present most traps caught at least some. The distribution of fish sampled by the traps was highly patchy on this spatial scale (variance to mean ratio: median = 4.0, range 0.5 to 69). Both of these characteristics, plus the lack of discrete cycling in abundance levels, support the idea that the pre-settlement mullids are organised into discrete schools that move unpredictably around the reef.

Catches from 4 to 6 rafts stationed along 2 kms of back-reef showed a similar trend in abundance as that among the light traps (Fig. 2.5b). *Upeneus tragula* were virtually absent at the station furthest into Watsons Bay (ST, Fig. 2.2). There was perhaps more consistency among rafts than among light traps as the major abundance peaks of mullids in early Jan. was present under all rafts, bar the one deployed at ST. However, little can be made of this comparison as the data were from two different years. More

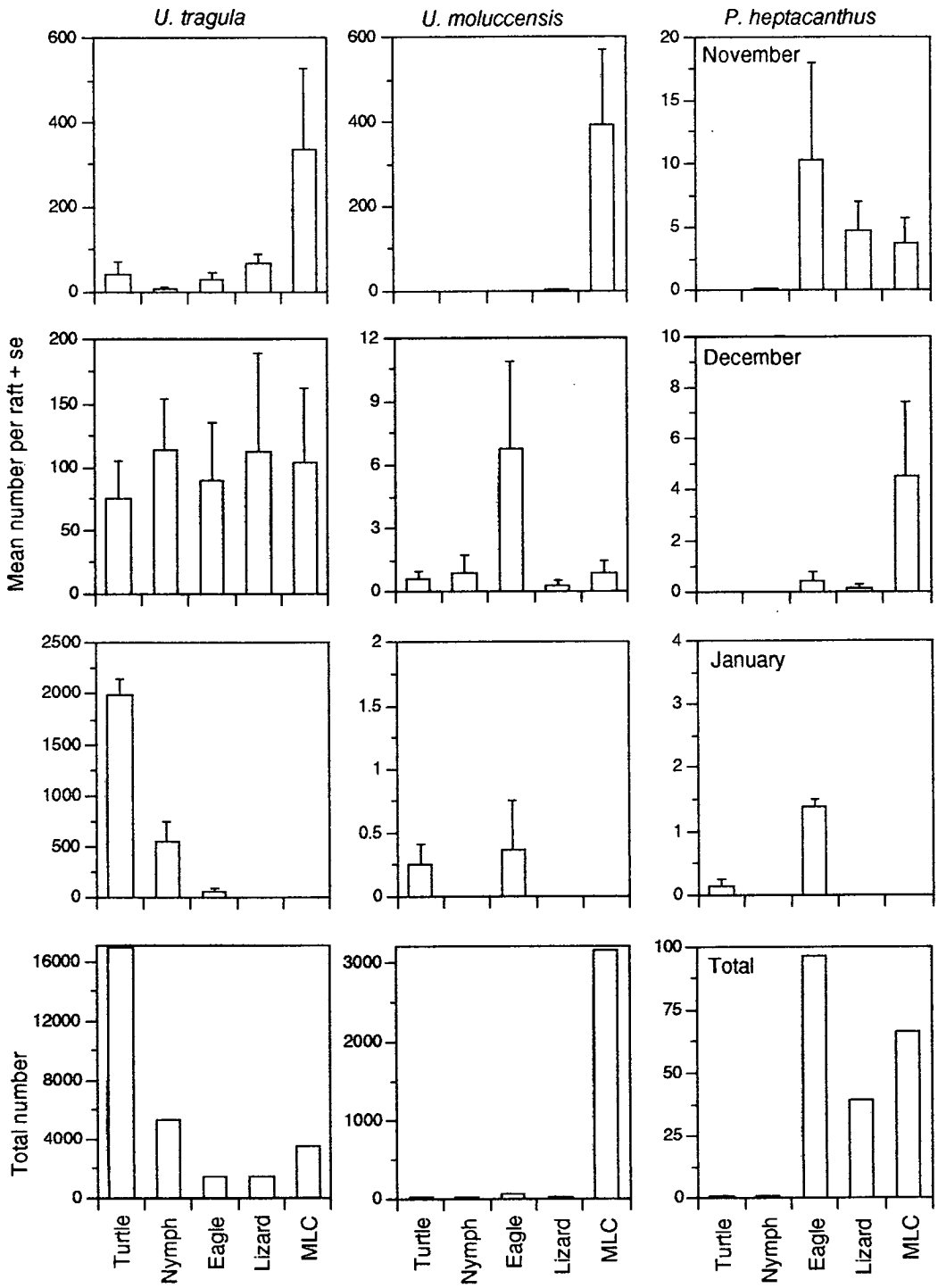

Figure 2.6. Abundance patterns of three mullid species at five stations across the northern Great Barrier Reef during Nov, Dec 1989 and Jan 1990. Error bars are standard errors.

importantly the variance to mean ratios were in the same order as that determined for the light traps (median of 21.3, range 1 to 237) further supporting the notion of organised schooling behaviour in these pre-settlement fish.

2.4.4 Cross shelf abundance patterns

Upeneus tragula was by far the most common pelagic-stage mullid caught under the aggregation rafts, representing on average 88 % of the catch within the GBR lagoon. This dominance was location dependent and ranged from 56 % at the station between Lizard Island and Carter Reef to 99.9 % at the inshore Turtle station (Table 2.2). In contrast, *U. tragula* only represented 9.7% of the pelagic mullids caught off the Outer Barrier. A cross-shelf trend in the abundance of *U. tragula* was evident when pooled over all months, with catches being highest at inshore stations and lowest at stations close to the Outer Barrier (Fig. 2.6). However, this trend is due to the extremely high numbers of *U. tragula* at the Turtle and Nymph stations during January. When abundances were examined for the 3 months separately, no consistent cross-shelf trend was apparent. The November abundance patterns were the inverse of the January patterns, and during December *U. tragula* were common at all locations across the shelf.

Table 2.2. Percentage composition of pelagic-stage mullids caught under aggregation rafts at sampling stations across the northern GBR.

Species	Turtle	Nymph	Eagle	Lizard	MLC	Outer Barrier
U. tragula	99.9	99.7	84.4	95.1	56.0	9.7
U. moluccensis	0.05	0.2	6.9	1.8	37.5	12.1
P. heptacanthus	0	0	8.3	2.8	6.0	0
P. barberinus	0.02	0	0.3	0.3	0.5	0
P. unidentifiable	0	0	0	0	0	78.2

Upeneus moluccensis was the second most abundant species of mullid that was associated with the aggregation rafts. When averaged over all months this species represented between 0.05 to 37.5 % of the overall catch (Table 2.2). *U. moluccensis* was commonest at the outermost lagoonal station during November (Fig. 2.6) when it reached abundances of up to 1054 fish under a single raft. Abundances were low in December with fish occurring at all stations, but were most abundant at the Eagle

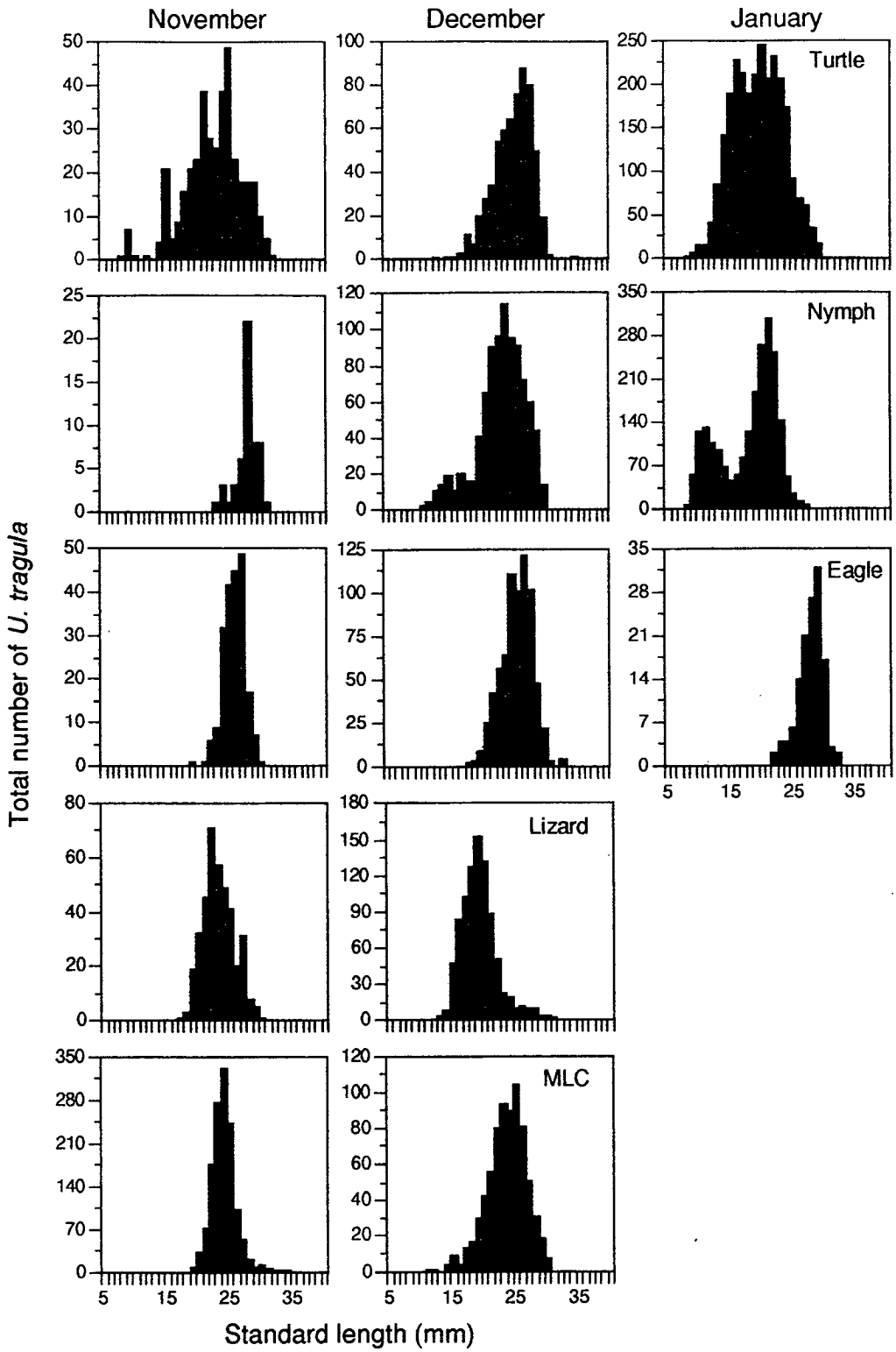

Figure 2.7. Size-frequency distributions of *Upeneus tragula* caught under rafts at five stations across the northern Great Barrier Reef during Nov, Dec 1989 and Jan 1990. No *U. tragula* were caught at Lizard or MLC (mid-way between Lizard Is. and Carter Rf) during the January sampling. Sample stations correspond to bold circles on Fig. 2.2.

station (6.75 per raft). Interestingly, large numbers were never present at the Lizard Island backreef, although they did occur 5 km NW of Lizard Island at the Eagle station.

Parupeneus heptacanthus was the most common of the reef-associated *Parupeneus* to aggregate under rafts. It regularly occurred in low numbers at or offshore from the Eagle station, occurring in highest abundances during November (Fig. 2.6). *P. heptacanthus* was rare or absent from the two inner-most stations during all of the three months of sampling.

Unidentifiable species of the genus *Parupeneus* made up the bulk of pelagic-stage fish caught under rafts off the Outer Barrier during the 1987 programme (78.2%, Table 2.2). No species that looked morphologically similar were found in the sampling inside the GBR lagoon. The absence of such specimens over four summers (1988 - 91) of intense sampling of the central lagoon off Lizard Island suggests this concentration of *Parupeneus* species is more likely to be a true locality difference rather than a temporal or chance difference. *Upeneus moluccensis* (12.1%) and *U. tragula* (9.7%) made up the remainder of the mullids caught off the Outer Barrier.

The size frequency distributions of *Upeneus tragula* showed no consistent trend across the shelf (Fig. 2.7). Distributions at the Turtle and Nymph stations were highly variable, at times showing marked bi- and multi- modality. The Turtle station had the widest size range for all three months of sampling, while the Eagle station had the narrowest, with a conspicuous lack of fish < 17 mm SL. The lack of evidence for a radiation of differentially sized fish from a discrete part of the shelf, and the presence of small individuals (< 15 mm SL) at all stations (with the exception of Eagle), suggests that *U. tragula* have spawning populations across the whole inner lagoon. The bimodality and often high abundance of small individuals in the inner stations suggests that the largest populations may be located in the shallow water seagrass beds inshore.

2.4.5 School structure

Visual observations from a moored 7m motherboat and tender, together with snorkelling observations showed that pelagic-stage mullids formed large neustonic schools. These schools were 'non-polarised' (Shaw 1970) and were seen swimming a meandering path in open lagoonal waters

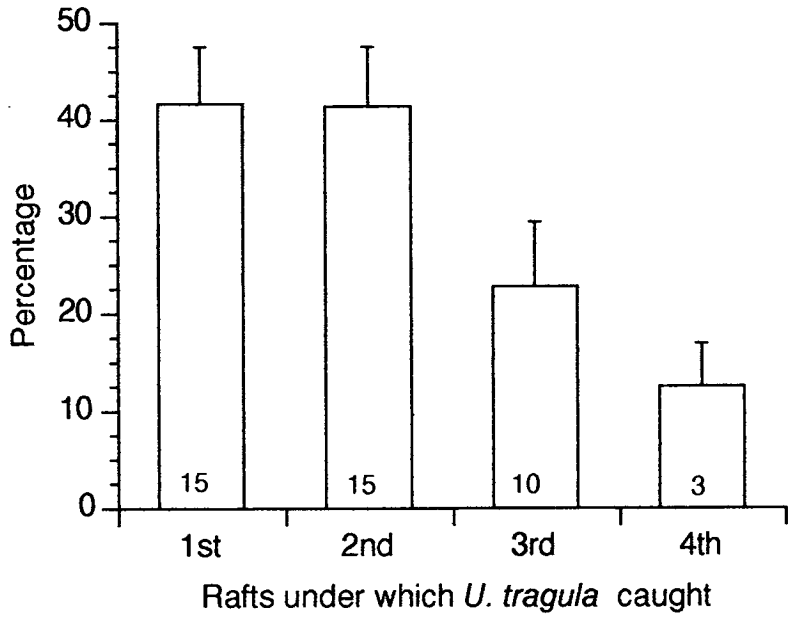

Figure 2.8. The influence of sampling disturbance as represented by the percentage of the total *Upeneus tragula* caught under particular rafts within a site. Raft 1, represents the mean percentage of fish caught under the first raft to have fish present within a site. The number of rafts within a raft category are also given e.g. on 3 occasions all four rafts (out of the four rafts sampled) within a site had fish under them. Note that these are mean percentages of a variable number of rafts so are not expected to sum to 100%.

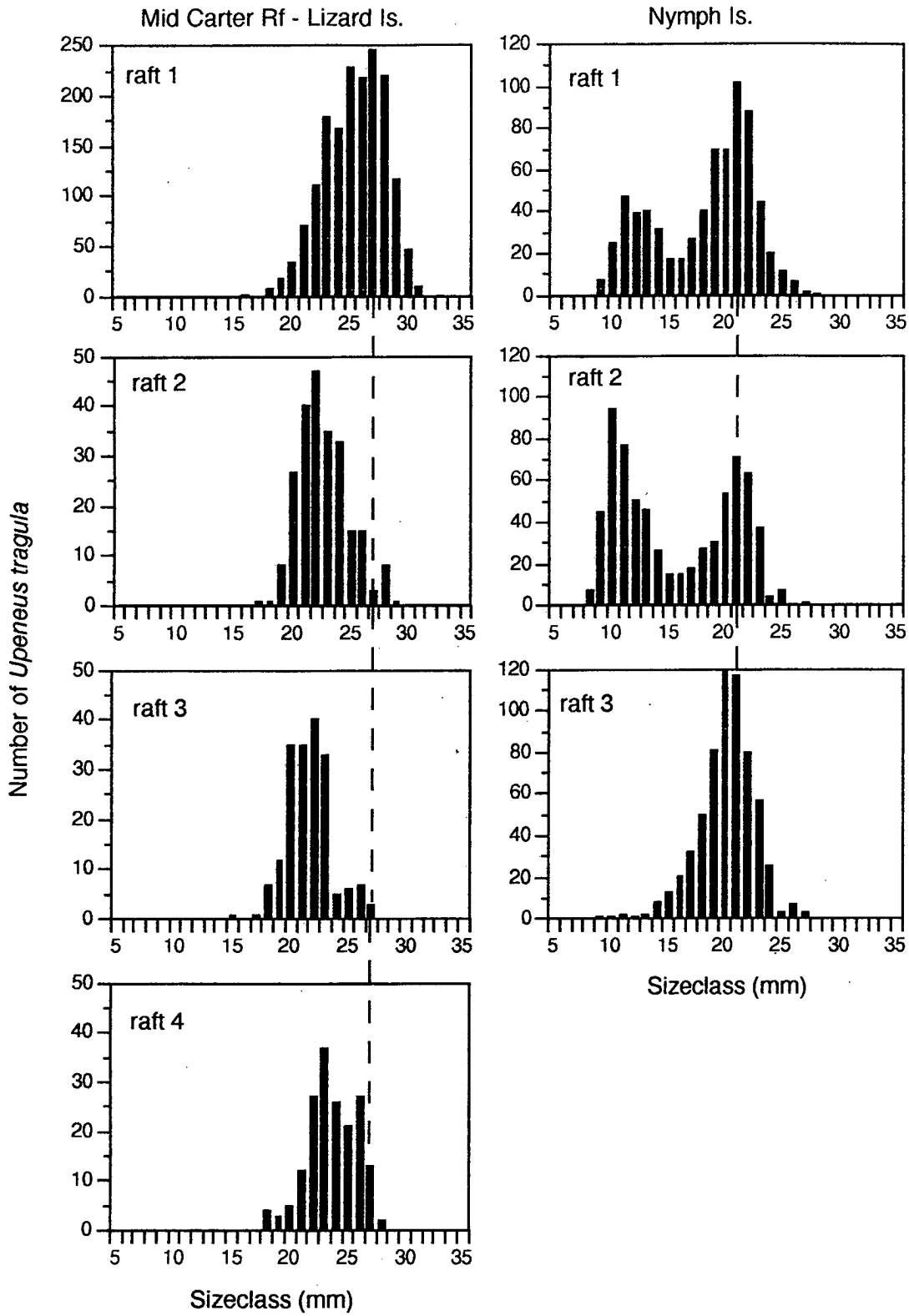

Figure 2.9. Comparison of the size-frequency distributions of consecutive raft samples of *Upeneus tragula* from two stations, sampled on 21st Nov 1989 and 17th Jan 1990 respectively.

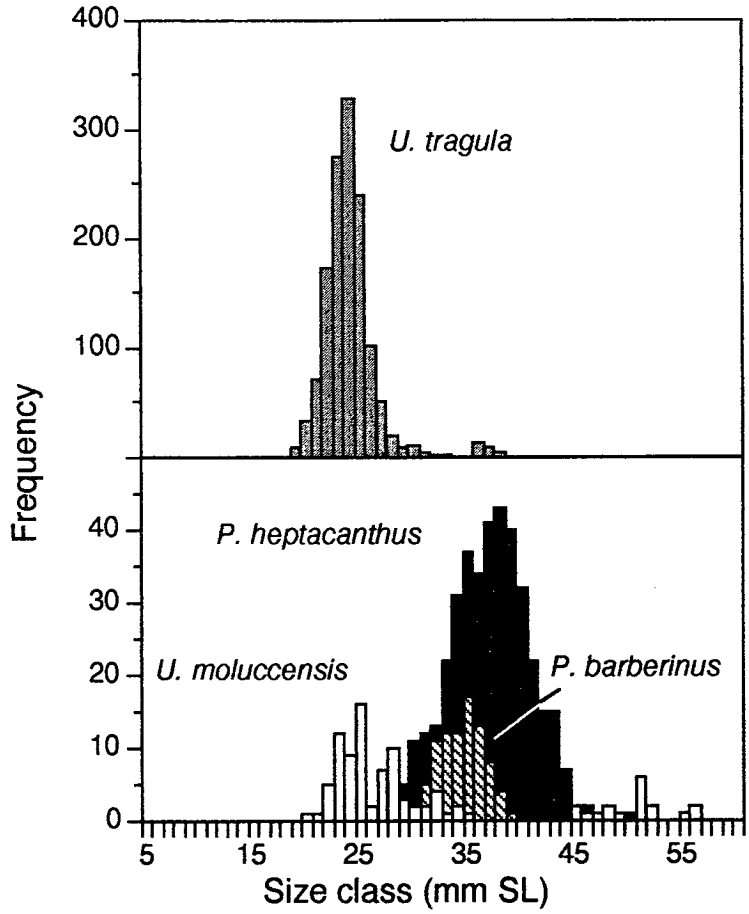

Figure 2.10. Species and size composition of a single purse-seine around an aggregation raft mid-way between Carter Reef and Lizard Island on the 31st October 1989. Genera are *Upeneus* and *Parupeneus*.

away from drifting objects. Underwater observations of these schools revealed that they comprised of a number of differing species that spanned over a wide size range. Estimates placed the number of fish in these schools in the thousands. This size range was similar to that displayed by purse-seine samples. Schools occupied from the surface to 2 - 3m depth.

Figure 2.8 shows the number of *Upeneus tragula* caught under *consecutive* rafts within a site, over all cross-shelf sampling. On average 41% of the fish collected at a site occurred under both the first and second rafts that had fish associated with them. Far fewer were caught under the remaining rafts, with only 12.5 % of the total fish collected for the site occurring under the last raft. Furthermore, on only 3 occasions were fish caught under the fourth raft. This pattern of capture, together with the fact that in 55 % of the cases the first raft to have *U. tragula* under it was not the first raft to be sampled in the series, suggests that sampling was not disruptive to the number of fish collected under each raft. The pattern may represent the sampling depletion of a single school that was associated with all the rafts. This suggests that rafts are not independent of one another and, by the nature of their aggregating effect, individually do not represent well the mean of the population within the site.

An examination of the size-frequency of consecutive raft samples found that the repeatability of patterns between consecutive rafts was highly variable. Figure 2.9 shows the size distributions of the catches of *Upeneus tragula* from consecutive rafts within a site at two stations across the shelf. The second raft at the Nymph station mirrored the bimodality of the first raft, while in the third raft sampled only the second modal population was present. Similarly, for a site mid-way between Carter Reef and Lizard Island, the size distribution from under the first raft was completely different from the subsequent 3 rafts (Fig. 2.9). This may suggest that at times there are multiple schools around the rafts within a site, rather than one large school associated with all rafts.

Figure 2.10 shows the composition of a typical purse-seine sample from the station mid-way between Carter Reef and Lizard Island during October 1989. This is assumed to represent the composition of a school. The size range of the school was broad, ranging from a *Upeneus tragula* of 19 mm SL to a *Upeneus moluccensis* of 56 mm SL. Similarly, for the individual species the fish length was highly variable. This was accentuated

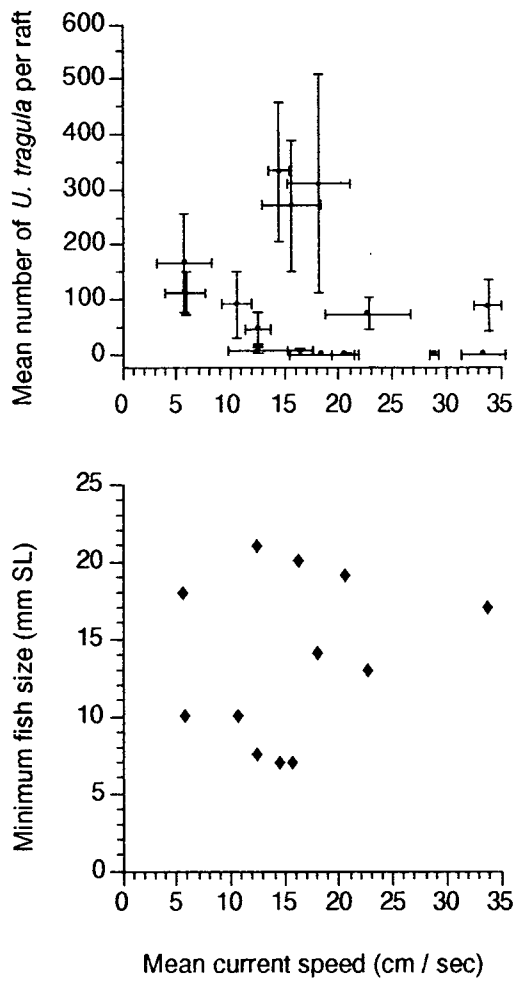

Figure 2.11. Relationship between current speed and number of *Upeneus tragula* caught under 8 rafts per station. The relationship was similar for the surface and 5 m drogues so data were pooled. Means for stations are plotted with standard errors (n = 4). The minimum size (mm SL) of *U. tragula* caught within a station versus current speed is also given.

by *Upeneus moluccensis* which exhibited a bimodal size-distribution, ranging from 20 to 56 mm SL.

The average current speed recorded at the surface and 5 m depth at each of the sampling stations during the aggregation period was not linearly related to the number of *Upeneus tragula* caught (Fig. 2.11). The highest number of *U. tragula* under a raft (1671) were caught in moderate currents (mean: 18.1 cm/s). This suggests that current speed, within the range measured, did not influence the sampling efficiency of the moored aggregation rafts. There was no obvious relationship between current speed and the minimum size of *U. tragula* caught at a site, although data is inconclusive on this point due to the lack of samples collected when water currents were above 23 cm/s. Together these data suggest that, within the range of currents measured, current speed has little effect on the number of fish in a school, and its size composition. It also emphasises the ability of even the smallest fish in the schools to maintain their position against moderate currents.

2.5 DISCUSSION

Spatial and temporal sampling by aggregation devices enabled the ecology of the late pelagic-stages of a reef fish family to be described in a detail not previously possible. This study shows that flexed mullid larvae, with a clupeiform body shape and clupeoid pigmentation, join schools consisting of a number of different mullid species, each having a broad size range. These schools contain fish of sizes capable of metamorphosis and settlement. This pelagic schooling life-stage can encompass 70% of the time the fish is in the planktonic environment (McCormick unpublished data), and occur close to and far from individual reefs.

The species composition of schools differ among localities across the northern shelf. In general, these schools were commonest in the main GBR lagoon away from reefs. Here they were prevalent throughout the summer months reaching very high larval biomasses, of up to 0.68 kg under a single 1 x 1 m aggregation raft (0.41 kg *Upeneus tragula*, 0.27 kg *U. moluccensis*). Schools also occurred in unpredictable pulses close around the Lizard Island fringing reef. The behavioural characteristics inferred from these observations emphasise the potential of the fish to determine their pelagic distribution patterns and their final reefal or inter-reefal destinations.

Two observations suggest that these schools are real structures rather than simply a product of the aggregation techniques employed. Firstly, mullids can be observed in tight polarised schools (Shaw 1970) meandering toward a stationary boat. When attacked by schooling piscivores, such as the mackrel tuna, *Euthynnus affinis*, these aggregations form tight balls typical of other schooling fishes (e.g. jack mackerel, *Trachurus symmetricus,* Schmitt & Strand 1982). Secondly, very high variance to mean ratios were obtain for both sampling techniques. Even though these are aggregation devices, low values could be expected if the fish were not aggregated, since all replicate samplers would attract a similar number of fish. Together these suggest that the fish from a light trap or raft sample represents a part or whole of a school of mullids, rather than simply a haphazard collection of individually navigating fish that have been attracted to the same object.

A broad size distribution was observed for all species in all samples collected. In the school examined in detail (Fig. 2.10), the largest *Upeneus moluccensis* was about three times the length of the smallest. This represents a 29-fold difference in wet weight (0.09 to 2.61 g). Hunter and Mitchell (1967), in a study of the ichthyofauna associated with flotsam off the Central American coast, found a similarly broad size range for the goatfish *Pseudupeneus grandisquamis* (26 - 54 mm SL). This suggests that the schools are dynamic and constantly recruiting and losing (via settlement) fishes. This conclusion does not conform to the optimal schooling model, where fishes of similar swimming ability are suggested to school together in order to accrue a hydrodynamic benefit (Lindsey 1978). Uniformity of size within schools is the norm for clupeoids (reviewed by Blaxter & Hunter 1982) where it is estimated that variation in length of fish in a school does not exceed 30% (Breder 1976). The present study has shown that schools are commonly multispecies aggregations each with its own broad size range. Overall a school may involve a size range from 7.5 mm SL (*Upeneus tragula*) to 65 mm SL (*U. moluccensis*). This represents a huge range in swimming ability and probably prey-type and -size specificities.

This schooling behaviour has important implications for the dispersal potential of mullids. Preliminary information I have collected on sustained swimming speeds using a flow tank suggests that a 23 mm SL *Upeneus tragula* can swim at speeds in excess of 30 cm/s (i.e. 13 body lengths/s) for

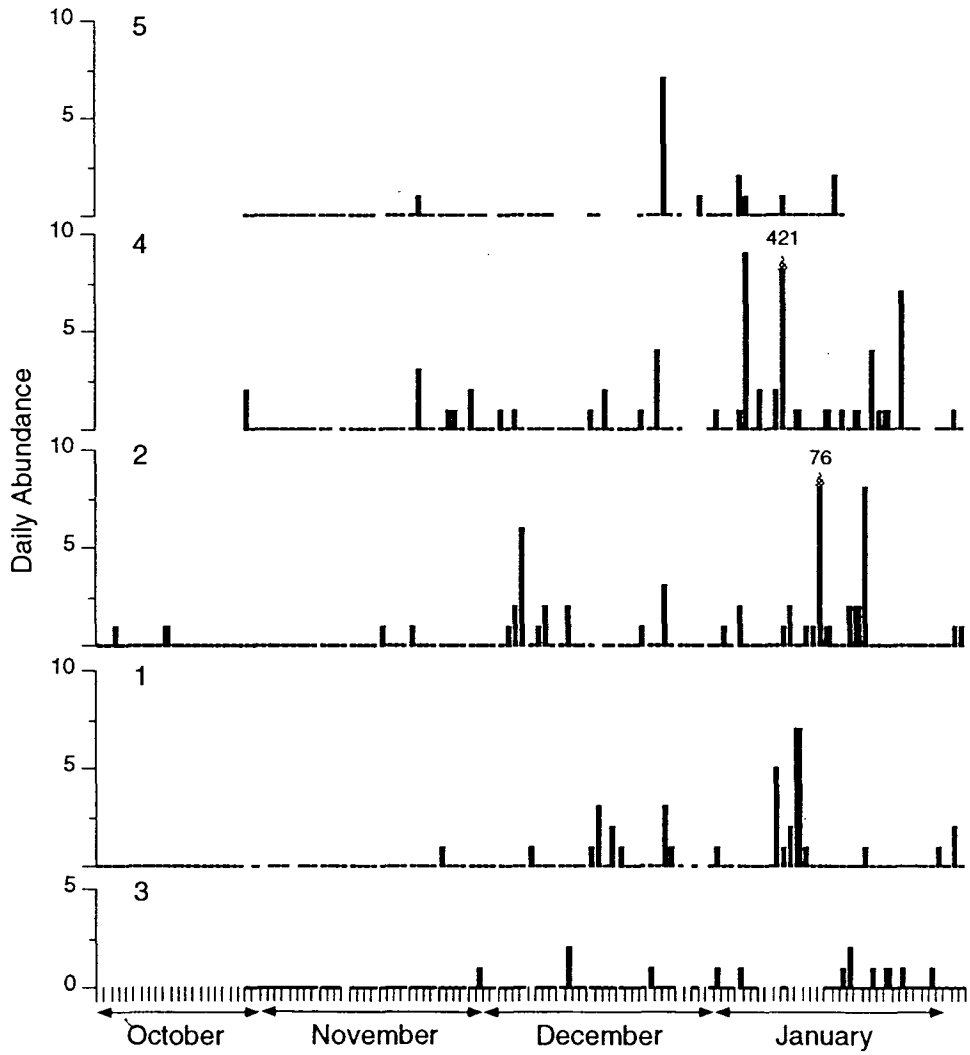

Figure 2.12. Daily catches of carangids from five light traps (1 to 5, Fig. 2.2 inset) at the front of Lizard Island during the 1986/87 season (from Milicich 1992).

a period of at least 2 hrs. This is extremely high compared to other published accounts (Wardle 1977, Beamish 1978), but swimming speeds of this order do not appear atypical for the late pelagic stages of many reef fish species (Stobutzki 1992, McCormick unpublished data). Currents measured in the surface waters across the northern shelf during 1989 / 90 averaged 18.0 cm/s, with a maximum of 38.0 cm/s. Thus, on physical ability alone, a goatfish mid-way through the pelagic phase could travel many kilometres against or across the current before settlement. However, to maintain schools comprising such a broad size and developmental range these schools must progress at the speed of the smallest and least capable fish (plus a function due to the hydrodynamic advantage of being in a school). If schools are relatively stable in size composition over the short term (as the general occurrence of a wide size range would suggest), then the rate of dispersal is more likely to follow that of the prevalent currents. To have an understanding of the potential for long distance dispersal it is necessary to know how long the school size structure persists, and the ontogeny of sustained swimming speed.

Trends in the abundance patterns of mullids across the shelf were found, however this was variable on an among-month basis. *Upeneus tragula* were found in high numbers at all positions within the lagoon over the 3 months of sampling, and beyond the Outer Barrier during 1987. Small individuals (< 15 mm SL) were present for at least one of the months at all of the stations, except the mid-shelf Eagle station. It is unknown whether this pattern is the result of populations spawning across the whole lagoon in the depths between the reefs, or alternatively whether it is a result of the active dispersal of larval schools. Unfortunately no data is available for the cross-shelf abundance patterns or reproductive periodicity of the adults in the seagrass (*Halophila* sp.) and *Halimeda* sp. beds between the reefs.

The cross-shelf raft sampling and the simultaneous light-trap programme around Lizard Island showed that pelagic-stage goatfish were present in high numbers on some part of the continental shelf over the whole summer. It was only periodically that they occurred in close proximity to the fringing reef of Lizard Island. In this regard they are similar to carangids in their pelagic distribution. Figure 2.12 shows the aperiodic occurrence of carangids caught in light traps at the front of Lizard Island, and displays a similarly poor correspondence among traps as found for mullids. Since the majority of mullids collected under rafts and in light traps

are inter-reefal as adults, this concordance between carangid and mullid distribution patterns is perhaps unsurprising.

The difference between the species composition caught by the aggregation devices and those settling to the reef further stresses the species-specific nature of the behaviour of pelagic-stage fishes. Only five species out of at least 11 potentially available for capture were present under rafts or in light traps. Caldwell (1962), in a descriptive study of NW Atlantic mullids, found a similar pattern of occurrence. Of the 4 species (in 4 genera) present in the area only 3 were ever caught as pelagic stages around night-lights. Species of the genus *Mulloidichthys* were not caught in either Caldwell's study, or in the present study. Furthermore, the temperate species *Upeneichthys lineatus*, common as pelagic juveniles in the coastal waters around north-eastern New Zealand during summer, was rare under floating objects (e.g. drift algae, Kingsford 1992), but was found attracted to night lights (Moltschaniwskyj 1989). This points to major differences in the ecology of mullids at the species level, involving energetics, physiology (sensory development) and behaviour (phototaxis and the tendency to aggregate around flotsam).

The close correspondence of the catch size-frequencies between the two aggregation devices suggests that abrupt behavioural changes are associated with the formation of pelagic schools. The behaviours associated with phototaxis and the attraction to flotsam appear to develop at approximately the same stage in the life-history. Whether this is simply a product of the schooling behaviour or an attribute of individual ontogeny requires a species-specific examination of the ontogeny of behaviour.

The high catches in the light traps is interesting given that all other evidence shows that mullids are diurnal. In a study of the vertical distribution of ichthyoplankton, Leis (1991a) used both bongo and neuston nets to sample day and night distributions. He caught no late-stage larval mullids in night tows, while mullids were prevalent in surface waters during the day. Furthermore, a study of *Upeneus tragula*'s visual system shows a high acuity during the pelagic phase suggesting a role as a diurnal rather than nocturnal feeder (McCormick & Shand In press). Tank observations support this (pers. obs.). At night mullids take up one of two positions in an aquarium. Some rest on the bottom, while an equal number sit obliquely in the top 3 cm of the surface waters fanning their pectoral fins. Both have a

mottled appearance and do not respond immediately to light. These behaviours were not size dependent.

That mullids occur in large numbers in light traps suggests that they enter the trap as a response to the activity of other organisms attracted to the light. I label this behaviour as secondary light attraction, as opposed to the direct photopositive response (primary light attraction) displayed by other groups (e.g. Pomacentridae, Milicich 1992). Observations from y-maze experiments (Milicich 1992) suggest that secondary light attraction may operate in other groups (e.g. Lethrinidae) that are also well-sampled by the light traps. However, further discrimination of these two modes of light attraction awaits further laboratory experiments.

The variability in spatio-temporal abundance patterns of mullids shown in this study, and the school composition and behaviour inferred, hint at the complexity of the pelagic life-history stage. To understand the link between pattern and process, studies are required on the behaviour and development (sensory and somatic) of the early life-stages and their interaction with the pelagic environment. It is only by investigating these late pelagic-stages, and the interaction of their physical ability and behaviour with current regimes, that we will develop an understanding of the importance of the pelagic phase in determining subsequent population dynamics.

Chapter 3

Development and changes at settlement in the barbel structure of the reef fish, *Upeneus tragula*

3.1 Synopsis

The development of the sensory barbels of the tropical goatfish, *Upeneus tragula*, was examined from their initiation early in the planktonic life through to the reef-associated juvenile stage. The structure of the barbel was examined histologically and found to represent an outgrowth of the gustatory (taste) system, composed of at least 50 % sensory tissue at settlement. Abrupt changes in morphology were found to be coincident with the 6 - 12 h settlement period: barbels rapidly moved forward along the hyoid arch to abut the dentary; the length of the barbels increased by up to 52 %; the epidermal layer increased, to comprise 75 % of the cross sectional area; and the mean size of the taste bud cells increased by up to 100 %. A strong relationship was found between barbel length and mean taste-bud size. This relationship was used to predict the mean taste-bud sizes for 237 newly-settled fish, collected as 12 samples over two recruitment seasons. Estimated mean taste-bud size varied significantly between samples. Experiments examined whether food availability or temperature of the water within the pelagic phase influenced the size of the barbels at settlement. Food availability influenced the relationship between barbel length and fish size. Slower growing fish had larger barbels relative to fish length than those that grew faster. Temperature was not found to dissociate fish somatic growth and barbel growth. Variability in sensory development at settlement, and the factors which influence it, may have important ramifications for the potential success of the fish once on the reef.

3.2 INTRODUCION

Many reef associated fishes undergo a settlement transition from a pelagic phase to a benthic-oriented juvenile and adult phase. This event involves a major change in the physical characteristics of the environment which the fish inhabits, and in the sensory stimuli perceived by the fish (e.g. McFarland & Munz 1975). Typically this change in habitat is coincident with a rapid change in the morphology of the fish, commonly termed metamorphosis (Youson 1988). The extent of these changes range from subtle changes in body pigmentation (e.g. pomacentrids) to a dramatic mobilization of cellular tissue and growth fields (e.g. pleuronectiformes, elopomorpha: Forstner *et al.* 1983, Pfeiler & Luna 1984, Pfeiler 1986).

The patterns of development which govern the fishes' body characteristics and behaviour at settlement are influenced by the environmental and biotic conditions within the pelagos (Hovenkamp & Witte 1991, McCormick & Molony 1992). These factors may also influence the fishes' sensory capabilities and the way they perceive their environment (McCormick & Shand in press). A demonstrable link exists between the development of an appropriate structure which enables the interactive response to the environment, and the ontogeny of behaviour (Balon 1985, Noakes & Godin 1988). Thus, by influencing the sensory development, events within the pelagic life-history phase have the potential to influence the behaviour of newly settled fishes, their subsequent patterns of growth, and mortality rates. However, the response of a developing sensory structure to environmental conditions can be complex (Pankhurst 1992). Detailed studies of the interaction between sensory development and the environment are required in order to understand the implications of the pelagic life-history on the survival probabilities of newly settling fish.

This study examined the changes that occur in one aspect of the sensory system of a reef fish during settlement. The system examined is the sensory barbels of the goatfish, *Upeneus tragula* (Family: Mullidae) which are outgrowths of the gustatory (chemosensory) system. These are extensively modified branchiostegal rays (Gosline 1984) which are not used in the pelagic life phase, but are important in the detection and manipulation of prey items once the fish has settled to the reef. In this chapter, the morphology and sensory capabilities of the barbels are described. Their development from the pelagic life-history phase to the

juvenile phase is examined and changes that occur at settlement are highlighted. The variability in the state of development of the barbels at settlement was examined from samples of newly-settled fish over two recruitment seasons as a measure of the fishes' potential sensory capabilities at settlement. Two experiments were undertaken to investigate whether food availability and temperature of the water column during the pelagic life-stage influences development, and whether these factors were able to explain the variability recorded in the field. Finally, the development of the barbel sensory system is compared to other aspects of the teleost sensory system.

3.3 MATERIALS AND METHODS

3.3.1 Collection

Pelagic stage *Upeneus tragula* were collected from 5 km west of Lizard Island on the northern GBR (Fig. 2.2), during the summer of 1990 to 1991. Fish were attracted to a series of 1 x 1 m plastic aggregation rafts moored for 2 h, and were caught with a 14 x 2 m plankton-mesh purse-seine (see Chapter 2 for details). These were carefully transported to the laboratory at the Lizard Island research station. Overnight, some fish changed pigmentation and settled to the bottom of their tanks. These fish were regarded as having been competent to settle at the time of capture. There were also fish that had started to attain the characteristic reef-colouration but which had not completely metamorphosed and settled. Individuals in fully- and partially-settled states were preserved for histological examination. Juvenile *U. tragula* were collected using a fence-net from the seagrass beds on the back-reef of Lizard Island, and preserved for comparison with pre-settlement and newly-settled fish.

3.3.2 Barbel development

Morphological development of the barbels was quantified using specimens preserved in either formalin (10% buffered in salt water) or formalin-acetic-acid-calcium-chloride (FAACC). FAACC consists of: formaldehyde (37%), 10 ml; glacial acetic acid, 5 ml; calcium chloride (dihydrate), 1.3g; water to 100 ml (L. Winsor, J.C.U. personal communication). Standard length, body depth at the pectoral fin and anal fin, head length, and barbel lengths were recorded. Measurements were made with calipers on fish larger than 20 mm SL, and for fish below this, with a calibrated video-microscope. The

video microscope was also used to measure the distance from the tip of the dentary to the end of the hyoid arch to quantify barbel migration throughout development. To quantify the internal development of the barbels a size range of fish (11 - 45 mm SL), preserved in FAACC, was examined histologically (see below).

3.3.3 Variability in taste-bud size at settlement

Fish were aggregated under rafts and caught as detailed previously. Four samples of *Upeneus tragula* were collected during the summer of 1990 to 1991 (Nov - Jan), and 8 samples the following summer (Nov - Dec). All fish captured were carefully brought back live to the laboratory. The morphological characteristics of those fish that settled overnight were measured as above. The mean taste bud size of the barbels from each newly settled fish was predicted from barbel length with a regression relationship. Analysis of variance was used to examine whether taste bud size differed among samples. The nature of the differences found were further examined by *a posteriori* Tukey's tests.

3.3.4 Effect of food availability and water temperature on barbel development

The influence of food availability and water temperature during the pelagic stage on the development of the barbels up to settlement was examined in two experiments. To examine the influence of food availability, 25 pelagic-stage *Upeneus tragula* (20 - 23 mm SL) were randomly placed in each of three 60-litre capacity tanks of four feeding treatments. The four feeding treatments consited of the following: fed *ad libitum*; fed once per day; starved every second day (fed *ad libitum* on alternate days); and starved 3d then re-fed *ad libitum*. Those fish which were to be fed more than once per day always had food in their tank during daylight hours.

In the temperature experiment, pelagic stage *Upeneus tragula* were randomly assigned to one of two temperature treatments: 25 °C or 30 °C. Three tanks (20 l) were set-up per treatment, each containing 20 fish. For both experiments, fish were fed *Artemia* sp. (Ocean Star strain) nauplii (36 to 48 h old). Fish which had undergone metamorphosis and settled to the bottom of the tank the night after capture were used as field controls. Tanks were examined for newly settled individuals at 06.30 hrs each day; these were removed, rapidly killed by cold shock and preserved for histological

examination. These experiments were run as part of two larger experimental studies, the details of which are given in Chapters 6 & 7.

3.3.5 Histology

To examine the morphology and distribution of taste bud pits, barbels of 3 newly-settled *Upeneus tragula* were preserved for scanning electron microscopy in gluteraldehyde and post-fixed with osmium. Samples were then taken through a alcohol dehydration series, critical-point dried and gold coated with a sputter coater. Samples were then scanned using an Etec Autoscan™ electron microscope.

Histological sections (6 μm) for light microscopy were made from 4 fish per treatment and stained in Azan. Sections were cut one-third of the total barbel length down from the tip. The area of the main tissue zones was quantified for the experimental fish, and those fish collected for ontogenetic examination using compound microscope linked to a video monitor by a video-analysis computer package (V-trace 2.2, E-Soft, AIMS). These tissue zones were: the area of epidermis; taste buds; nerve bundle; connective dermal tissue; and bone. For each fish, mean density of taste buds per millimetre circumference was calculated, and the mean taste-bud cross-sectional area determined by measuring the area of 8 taste buds. A pilot study had found that the measurement of eight taste-buds yielded an estimate with a precision of 0.05. Taste bud cells were recorded as being present in a particular section only if they possessed both a neural connection from the dermis and microvilli which penetrated the outer squamous epidermis through the taste bud pit.

3.3.6 Analysis

A principal component analysis was used to remove the confounding isometric size-component from the morphological data for the two experiments (Somers 1986), allowing analysis of the remaining shape component. Principal component 2 represented barbel allometric length in both experiments. This measure was compared among the four feeding and two temperature treatments using analysis of variance.

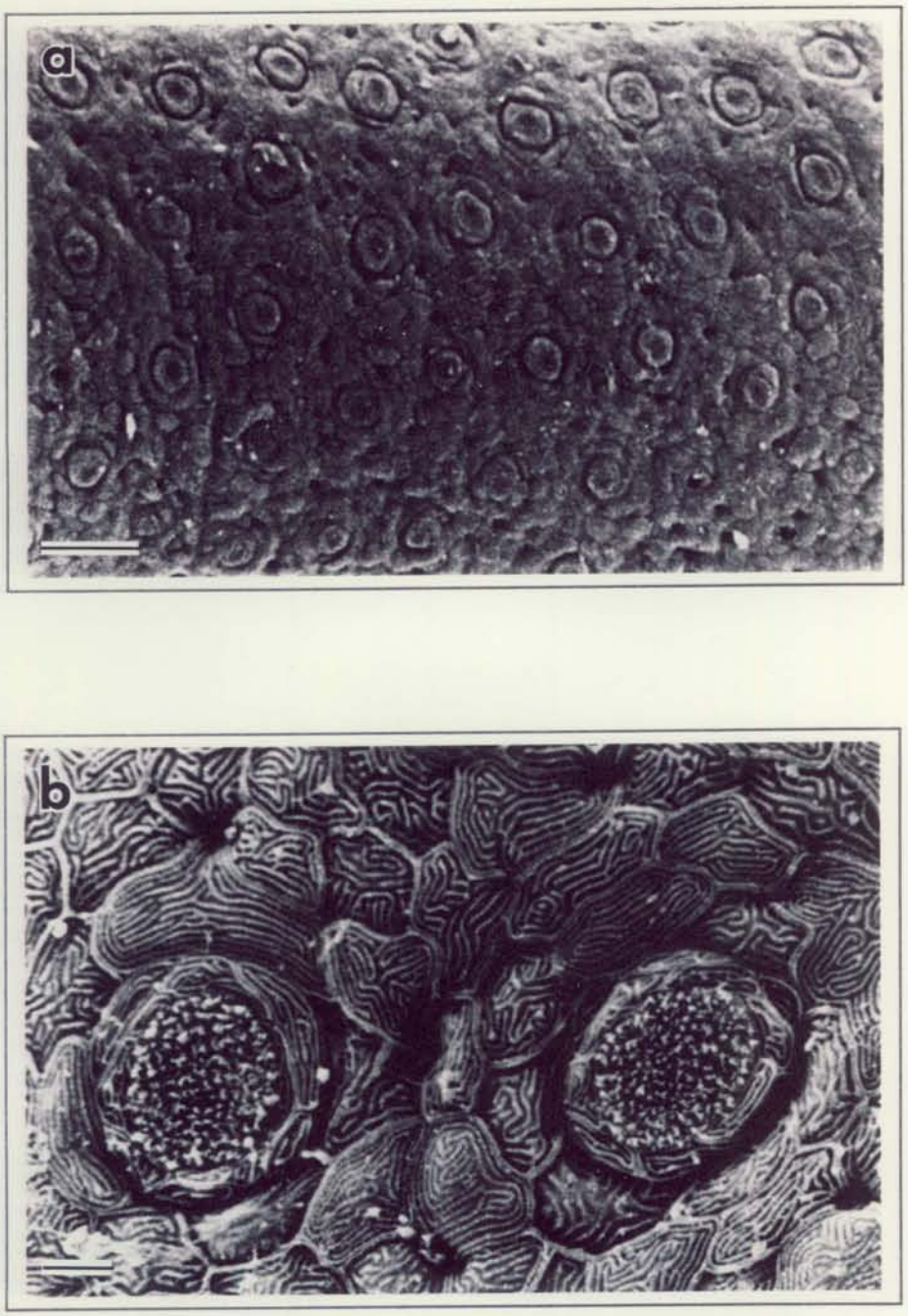

Figure 3.1. Scanning electron micrographs of the barbel surface one third of the length from the distal end. (a) Barbel surface showing the sculptured epidermal cells, and regular distribution of taste-bud pits (scale: 30 μm). (b) Details of the taste-bud pit showing collar cells and microvilli protruding from the pit. Note the pores of the goblet cells, between pits (scale: 10 μm)

3.4 RESULTS

3.4.1 Morphological description

The barbels of goatfish may be described simply as a rod of bone and cartilage supporting neural tissue and its associated external receptors. A scanning EM examination of barbels from three newly-settled *Upeneus tragula* showed that taste-bud pits were regularly arranged over the barbel surface (Fig. 3.1a), with an average distance between pits of 36 μm. The density of taste-bud pits (analysed as neighbour distances) differed significantly among four regions along the barbel, although *a posteriori* tests did not show significant differences (Table 3.1). The distance between pits was smallest at the tip and base of the barbel (34.1, 34.8 μm respectively), and greatest mid-way down the barbel and one-third from the tip (38.2, 38.4 μm respectively). The sensory pits are surrounded by a collar of modified squamous epithelial cells (Fig. 3.1b). Microvilli protrude from these pits. These are the terminal portions of elongated cells and make up the major part of the taste bud. The size of these pits differed significantly along the barbel (Table 3.1). The diameter of these pits were largest one-third the way along the barbel from the tip (15.1 $\pm$ 0.51 (se) μm), smallest at the base of the barbel (10.9 $\pm$ 0.18 μm) and of an intermediate diameter at the tip (13.2 $\pm$ 0.47 μm) and mid-way along the barbel (13.3 $\pm$ 0.41 μm). In addition to these pits, pores were haphazardly distributed over the barbel surface in similar densities to the taste bud pits (Fig. 3.1b). Examination of cross sections suggests that these pores do not penetrate deeply into the epidermis, and originate from glandular goblet cells, which have a mucus secretory function.

Table 3.1. Comparison of the (a) density (neighbour distance, μm) and (b) size (diameter, μm) of taste-bud pits on four regions of a barbel from the goatfish *Upeneus tragula* (tip, one-third barbel length from tip, mid-way along barbel, base).

	Variable	Source of variation	DF	MS	F value	*p*
a)	Distance between pits	Position	3	151.037	2.706	0.0485
		Residual	116	55.810		
b)	Pit diameter	Position	3	48.411	16.820	0.0001
		Residual	64	2.878		

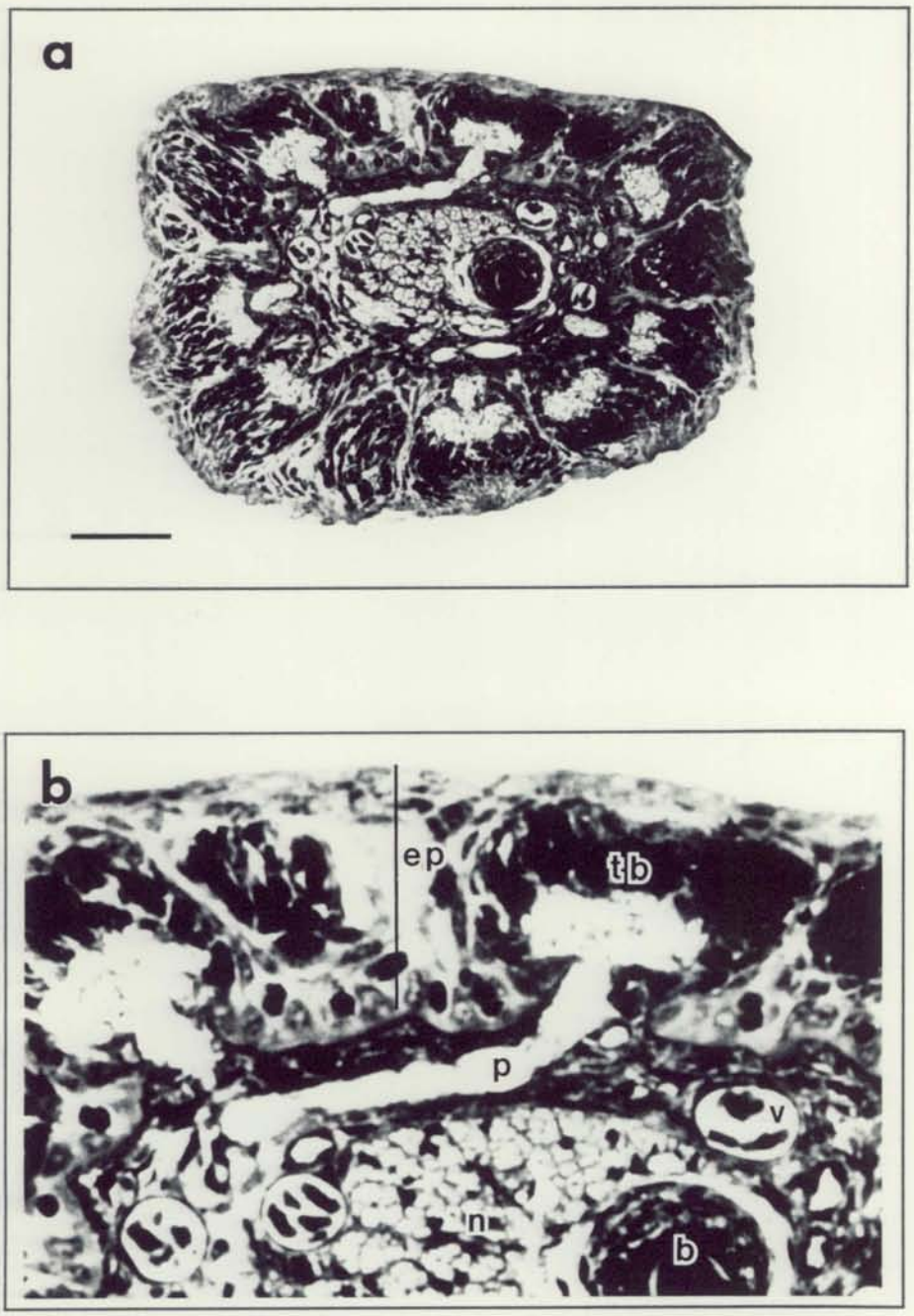

Figure 3.2. Cross-section of a barbel of a newly-settled *Upeneus tragula* showing the major structural regions. (a) Overview (scale: 45 μm). (b) Detail of the taste bud and papillar connection to the dermis. Key: b, bone; ep, epidermis; n, nerve bundle; p, papillar protrusion of dermis; tb, taste bud; v, blood vessel.

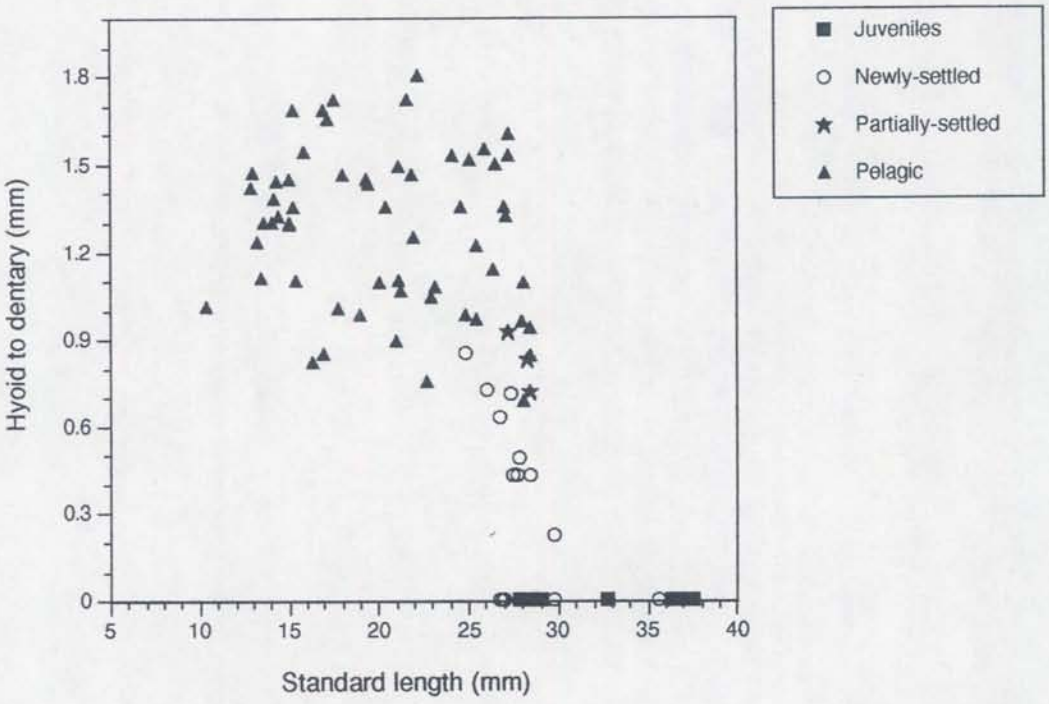

Figure 3.3. The migration of the barbels along the anterior certohyal measured as the distance of the hyoid bar from the dentary as a function of fish length. Juveniles, first-day settled, partially-pigmented and pelagic stage *Upeneus tragula* are indicated as per the legend. Symbols are the same for all other figures.

Table 3.3. Comparison of the mean predicted taste-bud sizes for each fish in twelve samples of *Upeneus tragula*, spanning two recruitment seasons (4 in 1990-91, 8 in 1991-92). Mean square error associated with regression is accounted for in mean square error of the ANOVA and Tukey's (HSD) tests. Grand means of each sample (μm^2), sampling dates and statistical grouping from Tukey's tests are also given.

Source of variation	DF	MS	F value	*p*
Sample	11	332782.8	4.956	0.0001
Residual	225	67142.5		

Sample	3	12	4	2	10	11	5	9	7	6	8	1
Date	8/1/91	16/12/91	10/1/91	5/12/90	8/12/91	11/12/91	9/11/90	3/12/91	15/11/91	13/11/91	18/11/91	11/11/90
Mean	1395	1365	1354	1324	1276	1273	1153	1138	1119	1094	1027	939
Tukey's Group												

Cross sections of the barbels showed that taste buds are located in a stratified squamous epithelium, separated from the dermis by a well defined basal membrane (Fig. 3.2 a, b). These flask-shaped taste buds are similar to those described by Reutter (1982) for the catfish genus *Ictalurus*. The taste bud is linked to the dermis by a papillar protrusion through which nerves connect the nerve plexus of the dermis to the basal cells of the taste bud. The dermis consists of connective tissue, large blood vessels, muscle cells and nervous tissue that emanates from the nerve bundle of the barbel. A large portion of the dermis is made up of bundles of nerve fibres, which represents a large branch of the facial nerve (Holland 1976). This nerve surrounds the ventral half of the supporting rod of bone and associated osteoblast layer (Fig. 3.2b).

3.4.2 Barbel development

The barbels develop as a modification of the anterior-most branchiostegal ray, articulating on extensions of the anterior end of the hyoid bar (anterior ceratohyal) (Gosline 1984). During larval development this modified ray migrates along the anterior ceratohyal, while at the same time the lower anterior ceratohyal extends forward. Figure 3.3 illustrates this forward migration as a measurement from the tip of the hyoid complex to the inside of the dentary. This distance is highly variable for fish of a given length prior to settlement. Furthermore, the evident drop in hyoid to dentary distance for the newly-settled fish, together with the fact that in 40% of these fish the hyoid abuts the dentary, suggests that the rate of the forward migration of the barbels increases markedly at the time of settlement (Fig. 3.3).

During the initial stages of barbel development they are attached by a membrane to the ceratohyal. The stage at which this membrane breaks, and consequently the time at which the barbels were potentially capable of functioning, was found to be highly variable both in terms of the size of the fish and barbels. Barbels ranged in size from 0.9 to 2 mm long when first detached, while fish ranged from 10 to 22 mm SL.

During the pelagic phase the barbels exhibited a linear growth with fish length (Fig. 3.4, Barbel length= 0.183 SL - 1.283, r^2 = 0.860, p < 0.0001, n = 180, zero values excluded). Barbels grew rapidly during the metamorphosis associated with settlement; a process which occurred overnight and took between 6 and 12 hr in the laboratory. This growth spurt

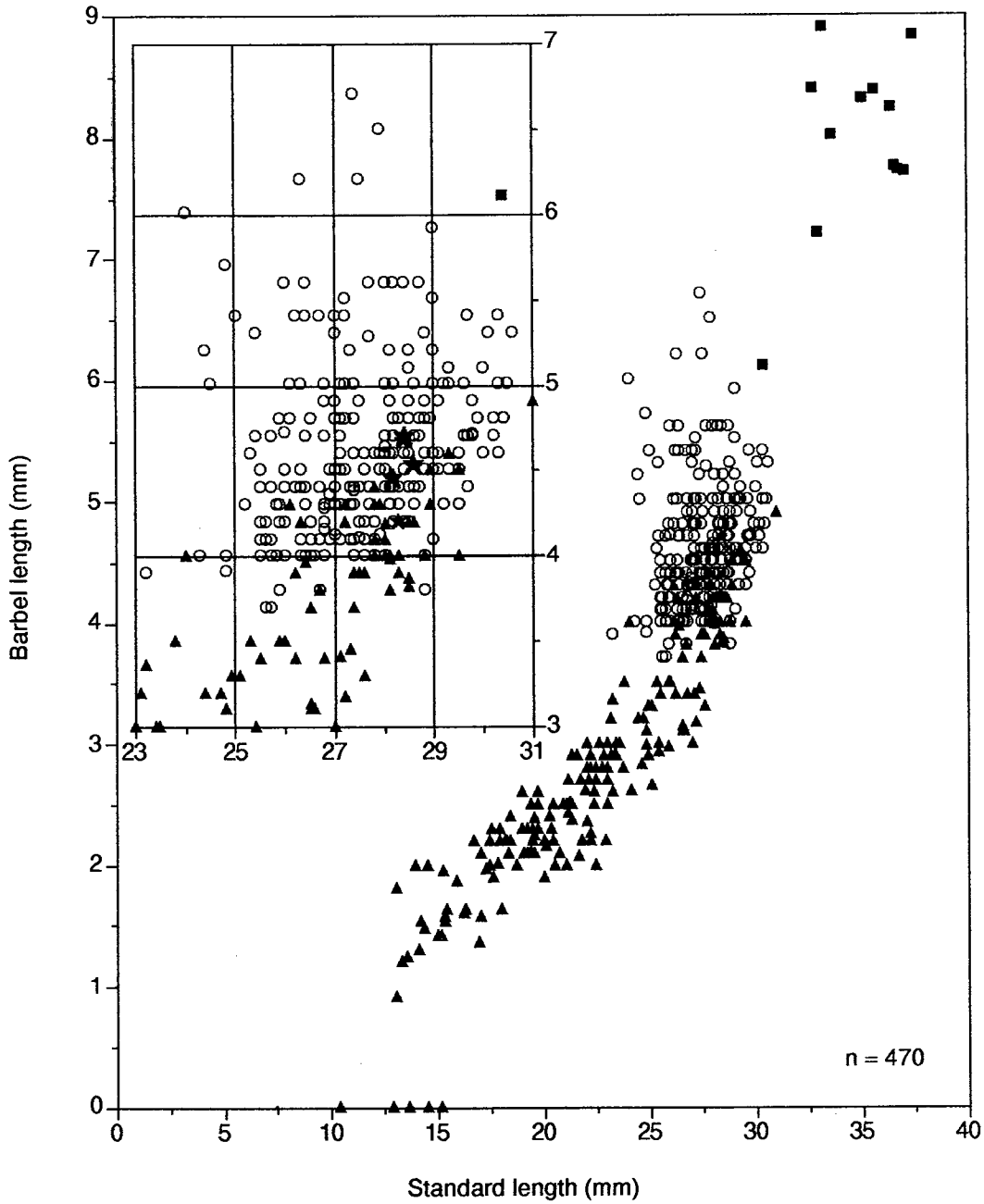

Figure 3.4. The development of the barbels of *Upeneus tragula*. Inset shows an expanded section of the graph where developmental stages of the fish overlap (Symbols as for Fig. 3.3).

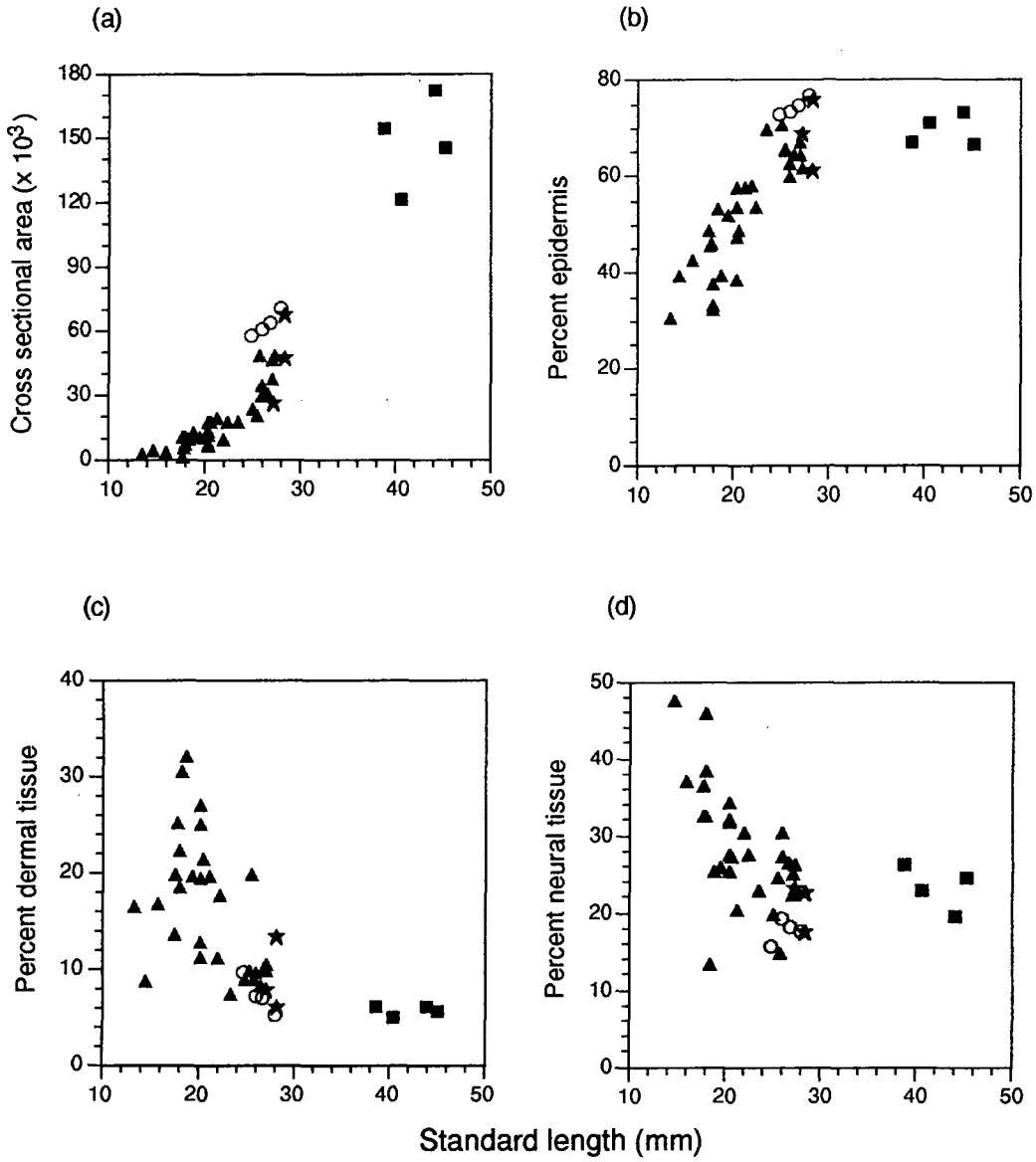

Figure 3.5. Histological changes in the barbels with fish length for *Upeneus tragula.* (a) Changes in the cross-sectional area of the barbels (one third of their length from the tip). (b) Change in the percentage of cross-sectional area of the barbels composed of epidermal tissue. (c) Change in the percentage of cross-sectional area of the barbels composed of dermal tissue. (d) Change in the percentage of cross-sectional area composed of neural tissue. The four developmental stages are shown (symbols as for Fig. 3.3).

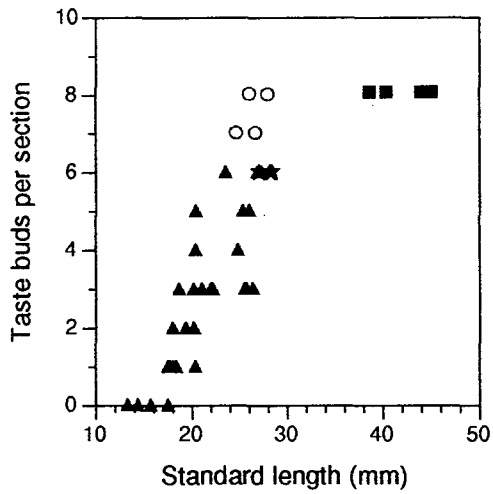

Figure 3.6. Change in the number of taste buds per cross-section through *Upeneus tragula* barbels. The four developmental stages are shown (Symbols as for Fig. 3.3).

may represent an increase in length of up to 52% in 12 h. After settlement, barbel growth returns to approximately the pre-settlement rate (Fig. 3.4, Barbel length = 0.163 SL + 2.298, $r^2 = 0.223$, $p < 0.001$, $n = 12$). Barbel lengths from the partially-settled fish support the notion of rapid growth at settlement, being midway in length between the mean length of pelagic fish and the mean of the newly-settled fish (when calculated for fish of settlement size) (Fig. 3.4, inset).

A rapid increase in cross-sectional area of the barbel was found to occur at settlement (one-third the length from the tip) (Fig. 3.5a). This was mirrored by an increase in the percentage of the total cross-sectional area made up of epidermis (Fig. 3.5b). The percentage contribution of the epidermis to the total cross-sectional area of the barbel exhibited an asymptotic increase which peaked at settlement at approximately 75% (Fig. 3.5b). This contribution appears to remain relatively constant in the juvenile stage.

Concurrently, the neural layer displayed an asymptotic decrease, with the point of inflection at approximately 18 %, corresponding to settlement (Fig. 3.5c). The percentage contribution of dermal tissue displayed a similar trend, maintaining a constant value of 6 % after settlement. The amount of dermal and neural tissue in the barbel prior to settlement was highly variable (Fig. 3.5c, d). This variability appears to be reduced after settlement, although this may also be a function of the smaller sample size.

The increase in the contribution of the epidermal layer to the cross-sectional area of the barbel is largely due to the contribution of the taste-bud cells. The number of taste buds present in a 6 micron section increased linearly to a maximum of 8 at settlement. The number was then maintained in the juvenile stage (Fig. 3.6). The size of the taste-bud complexes increased with standard length and displayed a similar trend to the change in barbel size. Taste buds increased their size by up to 100% during the 6 - 12 h period of settlement, reaching an average size of 1663 μm^2 (46 - 81 μm long axis, 23 - 58 μm short axis) (Fig. 3.7).

Both epidermal area and the mean size of the taste-bud cells were highly correlated to barbel length, while the contribution of the neural bundle to cross-sectional area and taste-bud density were only poorly related to barbel length (Table 3.2). This high correlation between taste-bud

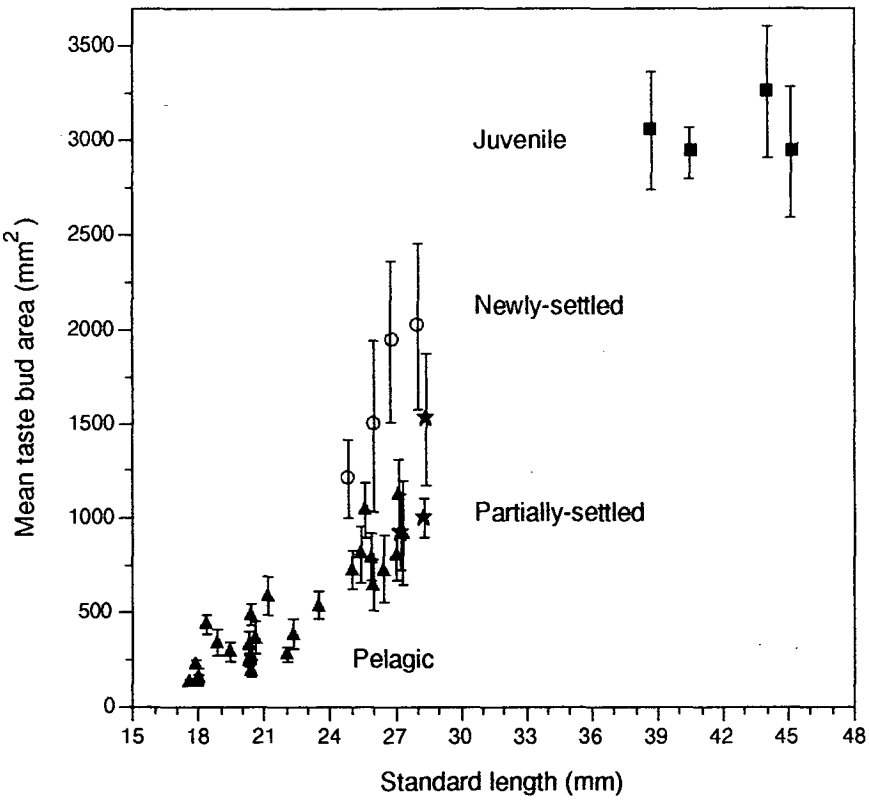

Figure 3.7. Change in the area of the taste buds with fish length for the four developmental stages of *Upeneus tragula* examined. Means and 95% confidence limits are given (n = 8). Symbols as for Fig. 3.3.

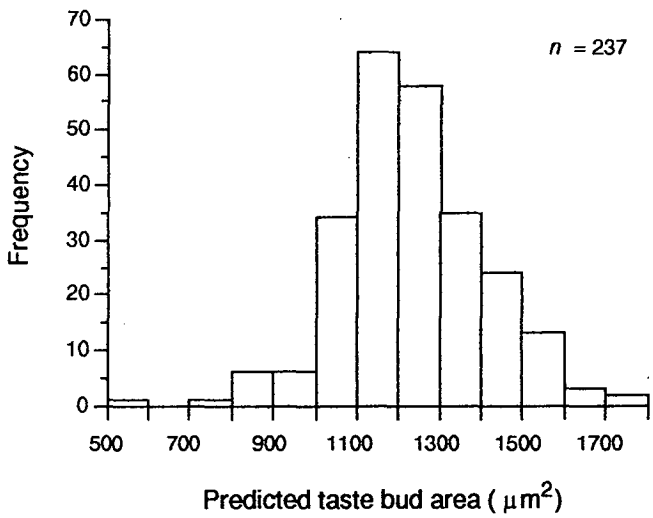

Figure 3.8. Size distribution of predicted mean taste-bud areas for *Upeneus tragula* that settled the night after capture. Taste-bud areas are predicted from the length of the barbels by the equation given in Table 3.2.

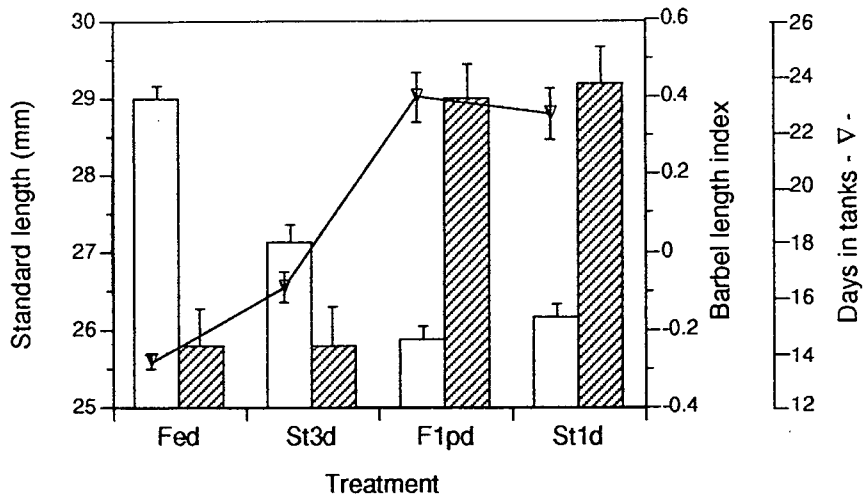

Figure 3.9. Results of an experiment to examine the influence of feeding history in the pelagic stage on sensory development at settlement. Four feeding treatments are: Fed, fed *ad libitum*; St3d, starved for 3d then re-fed *ad libitum*; F1pd, fed once per day; St1d, fed *ad libitum* every second day, starved on alternate days. The fish length (plain bars) and allometric barbel length of the fish (principal component standardised, hatched bars) are given. The time that fish were in the four treatments before settling is represented as a line plot. Error bars are standard errors.

area and barbel length enables an examination of the potential sensory capabilities of a large number of fishes at settlement.

Table 3.2. Relationship between barbel length (BL) and four aspects of barbel microstructure for *Upeneus tragula*

Dependent	Equation	r^2	*p*	*n*
Mean taste bud area (mm^2)	407 BL - 608	0.9357	0.0001	36
Taste bud density (no. per 0.01 mm)	2.088 BL - 0.226*	0.4000	0.0001	36
Epidermal area (mm^2)	13660 BL - 24707	0.9080	0.0001	41
Neural bundle area (mm^2)	918 BL + 133	0.5918	0.0001	40

* juveniles excluded

3.4.3 Variability in taste-bud size at settlement

There were significant differences between the mean taste-bud sizes, predicted from the relationship between taste-bud area and barbel length (Table 3.2), for the 12 samples of newly settled fish (Table 3.3). Mean taste-bud areas among samples ranged from 939 to 1395 μm^2, while overall they ranged from 532 to 1713 μm^2. The distribution of taste-bud sizes represented a normal distribution with a coefficient of variation of 21.1 % (Fig. 3.8).

3.4.4 Feeding and temperature experiments

The allometric size of the barbels was found to be affected by the four feeding treatments (Table 3.4a). Barbels were largest on those fish which had received the least food (starved every second day, and fed once per day) (Fig. 3.9). This effect was consistent among the three tanks per treatment (Table 3.4a). Food availability also influenced the internal structure of the barbels (Table 3.4b, c). The four feeding treatments did not affect the percentage of the cross-sectional area of the barbel comprised of epidermis, however, as a whole the feeding treatments did have significantly more epidermis than the field controls (Tukey's tests). The mean taste-bud size of the fishes significantly differed among treatments, with the "starved every second day" treatment having the largest taste-buds, the fully-fed treatment having the smallest, and all the treatments having significantly larger taste-buds than the field-control (Fig. 3.10, Table 3.4c).

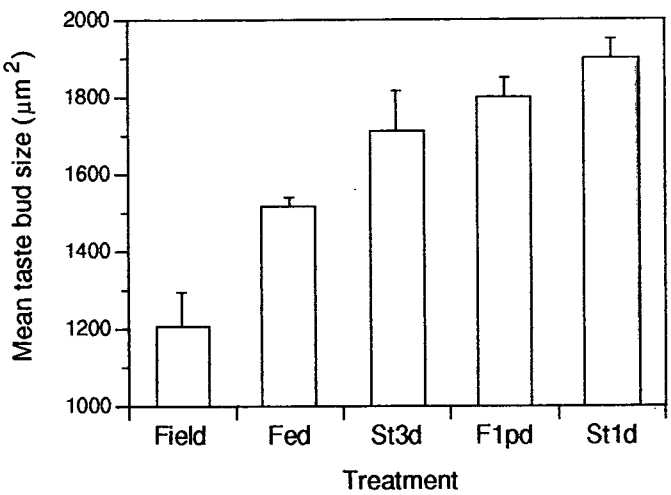

Figure 3.10. Mean taste-bud size of barbels from four *Upeneus tragula* per treatment, for the four feeding treatments and a field sample.

For both the taste bud size and epidermis area, the ranking of the four feeding treatments was similar to that found for barbel length. The density of taste bud cells in a 6 micron section did not significantly differ among the four treatments or field control (Table 4d), but ranged from an average of 6.5 / mm circumference in the fully-fed fishes, to 8.25 / mm for fishes in the starved-every-second-day treatment.

Interestingly, there was a negative relationship between barbel size and body growth-rate. Fishes in the fully-fed treatment had the highest growth rate, settled at the largest size, but had the smallest barbels (Fig. 3.9).

Table 3.4. The influence of food quantity and temperature in the pelagic stage on the length and microstructure of *Upeneus tragula* barbels at settlement. A. Comparison of barbel lengths from fish held within three tanks in four feeding regimes (diet). PCA was used to remove the confounding size component; PC 2, represented barbel allometric length, and accounted for 18% of the variation in the data set. B - D. Comparisons of various microstructural features among four feeding treatments and a field control (n = 4 fish). B. Epidermal area expressed as a percentage of total cross sectional area. C. Mean taste bud sizes, calculated from 8 taste buds areas per fish. D. Taste bud density, expressed as number of taste-buds within a section divided by cross-section circumference (mm). E. Comparison of barbel lengths from fish in 3 tanks at two temperature regimes (25, 30 °C). PCA used as for A, and represented 5% of variation in the data set.

	Variable	Source of variation	DF	MS	F value	p
A)	Barbel length	Diet	3	5.237	13.39	0.002
	(principal	Tank (Diet)	8	0.391	0.76	0.635
	component 2)	Residual	217	0.512		
B)	Percentage	Diet	4	173.050	9.59	0.0005
	epidermal area	Residual	15	18.050		
C)	Taste bud	Diet	4	299996	15.32	0.0001
	size (μm^2)	Residual	15	19584		
D)	Taste bud	Diet	4	2.125	0.92	0.476
	density (No./ mm)	Residual	15	2.300		
E)	Barbel length	Temperature	1	0.059	0.33	0.598
	(principal	Tank (Temp)	4	0.180	0.66	0.624
	component 2)	Residual	84	0.274		

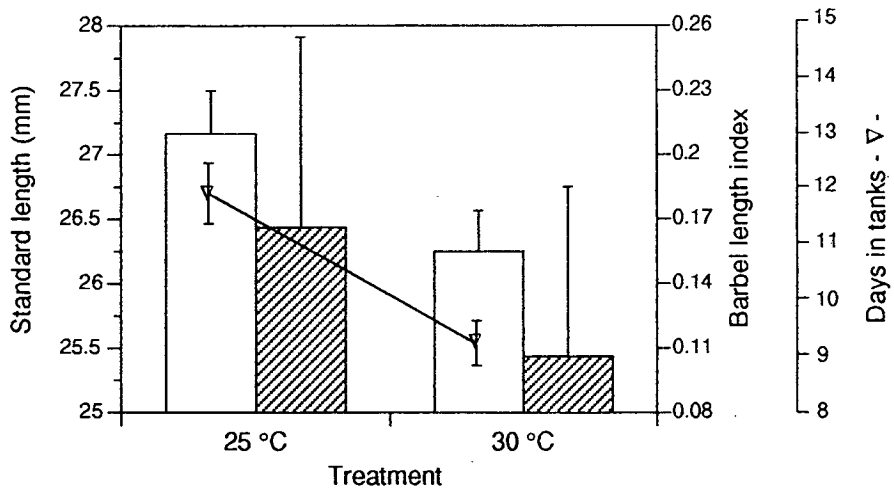

Figure 3.11. Results of an experiment to examine the influence of water temperature within the pelagic stage on sensory development at settlement of *Upeneus tragula*. Standard lengths (plain bars), allometric barbel length (hatched bars), and number of days that fish were in tanks before they settled is given for the two temperature treatments.

There was no effect of temperature on the growth of barbels at the life-history stages used in the experiment (Table 3.4e), and the dissociation of growth of the body and barbels evident in the feeding experiment did not occur (Fig. 3.11). Both allometric barbel length (i.e. PCA standardized) and standard length were smaller in the 30 °C treatment than when reared at 25 °C, although this difference was not statistically significant (Table 3.4e). Fish in the 25 °C treatment did, however, take significantly longer to settle than in the 30 °C treatment (Fig. 3.11).

3.5 DISCUSSION

The present study emphasises the complex nature of sensory development and the potential importance of the sensory status of an individual at settlement. Four important points emerge from the results. The barbels of mullids are rich in chemosensory structures. These sensory barbels show a brief period of rapid growth and differentiation which coincides with the transition from the pelagic to benthic life. The degree of development at settlement varies amongst cohorts of settling mullids. This variability in development can be governed by the feeding history within the pelagic, through the influence of food on somatic growth rates and the timing of settlement. These observations have a number of important implications for our understanding of the development of sensory systems, in particular the relationship between fish size and sensory development.

Reviews of larval-prey/predator interactions stress the importance of the size of fish larvae in determining survival, and the multiplicative advantages of a large body size through a positive feedback loop (e.g. Pepin *et al.* 1987, Yin & Blaxter 1987, Miller *et al.* 1988, Fuiman 1989, Margulies 1989). In such arguments sensory development is functionally tied to fish size. Generally, researchers have approached these questions pertaining to body size and development from a population perspective. However, when we look in detail at the individuals which make up the population at a particular ecological stage, as in this study, we find considerable variability in development. Furthermore, the sensory state of an individual was found to be under endogenous control with no feedback to survival schedules. Thus, on an individual basis, fish length was not necessarily a good predictor of sensory capability.

Experimental manipulations of feeding history through to settlement found that fish with the largest body size at settlement had the least

developed sensory system. Given that the ability of a fish to detect and capture prey and avoid predators will be related to both sensory and somatic development, there will be a survival trade-off between the speed at which a fish gets through the pelagic phase and its level of sensory development at settlement. What becomes important then is how ecological constraints, such as disease and predation, act as filters on the variability in the attributes of the population reaching the next developmental state.

Current research suggests that the mechanism behind this developmental response to varying biotic and environmental conditions may be similar for different elements of the teleost sensory system (e.g. Secor & Dean 1989, Pankhurst 1992). This study found that food availability within the pelagic stage influenced the development of the barbels, while water temperature over an ecologically meaningful range had little effect. The length of the barbels and size of the taste buds were related to the length of time the fish had been pelagic, rather than the amount of food obtained. Experiments on bones within the labyrinth system have yielded similar results. A number of studies have found that varying levels of food availability will cause the dissociation between otolith and somatic growth rate (e.g. Campana 1983, Reznick *et al.* 1989, Secor & Dean 1989). Some studies have found that temperature will not influence this relationship (e.g. Marshall & Parker 1982, Neilson & Geen 1982). It is possible that if a greater range of temperatures had been used, so as to straddle the optimal temperature for somatic growth, then a breakdown in the relationship between somatic growth and otolith growth may have occurred (e.g. Mosegaard *et al.* 1988). However, this would have been meaningless in determining whether or not the range of temperatures at settlement influenced the barbel development, since only a restricted range of temperatures occur in the water column at sampling location over the main recruitment period (25 - 30 °C, Chapter 7). Recently, Pankhurst (1992) found a similar relationship between somatic growth and visual development. Fish kept under poor feeding conditions had very large eyes relative to their body size compared to field caught fish. The growth and development of the barbels and eyes are likely to follow the model proposed for otoliths in being controlled by an endogenous rhythm that is modified under extreme conditions (e.g. Campana 1983) by the same factors that influence somatic growth (Campana & Neilson 1985, Secor & Dean 1989). The growth and differentiation of sensory tissue has a

conservative response to environmental and biotic fluctuations, being an essential part of the developing body maintenance network.

In this conceptual framework metamorphosis of the sensory barbels does not occur in direct response to the change in environmental cues associated with settlement, but rather is endogenously controlled and simply coincident with the shift between pelagic and benthic life. The magnitude of the sensory changes that occur emphasises the potential importance of settlement as a life-history transition. The changes which occur at metamorphosis and the potential for high mortality have led some researchers to refer to settlement as a "critical period" (e.g. Blaxter 1988). In *Upeneus tragula*, metamorphosis represented a rapid change in the structure of the hyoid arch and barbels. Overnight the barbels completed their migration along the hyoid arch to abut the dentary. Simultaneously there was a dramatic increase in barbel length which was coincident with an increase in the size of the taste buds. McCormick & Shand (In press: Appendix 1) found that a complimentary change in the morphology of the retina occurs in *Upeneus tragula* during settlement. An unusual double-layer of photoreceptive cone cells and a high ratio of processing cells to receptor cells was present in the retina of pelagic *U. tragula*. This configuration resulted in a high visual acuity. During the 6-12 h period over which settlement occurred the cone layers inter-meshed to form a single layer, and information processing efficiency was greatly reduced (McCormick & Shand In press, Shand In press). These changes in the barbel and visual systems, together with marked changes in the pigmentation are concurrent with an equally marked change in behaviour. At settlement *U. tragula* changes from being an active planktonic predator that uses its visual and lateral line system to detect food items, to a benthic micro-carnivore which uses its barbels to aid in the detection and capture of prey.

In many cases there exists a strong correlation between the structural and functional development of sense-organs and changes in the behaviour of the developing fish, which coincide with ontogenetic shifts in their ecology (Balon 1985, reviewed by Noakes & Godin 1988). Noakes & Godin (1988) note that in the herring, *Clupea harengus*, an area of high visual acuity differentiates on the retina only at or shortly after metamorphosis, following which the juvenile fishes feed more actively on zooplankton. Some of the most dramatic demonstrations of correlations

between structural and functional changes have been for pleuronectiforms. Unlike most families of reef fish, these fish undergo major remodelling during the metamorphosis associated with settlement (Ryland 1966, Neave 1984, Rao and Finger 1984, Markle *et al.* 1992). The present study is one of the few detailed examinations of the changes that occur during the settlement of a reef associated fish. The magnitude and rapidity of the changes (over hours rather than days) that occurred have not previously been documented. However, unpublished data suggests that although some changes in the sensory systems of most reef fish are likely to occur during the settlement transition, these may be accentuated in the goatfishes (J. Shand pers. comm.).

The sensory development of a fish at settlement is important since the interaction between the sensory capabilities and behaviour will determine recruitment patterns through site selectivity. However, this study stresses that the interaction between the ontogeny of the sensory system and the environment are complex. An increasing body of literature on otolith microstructure suggests that there is a three-way interaction between the environment, the sensory structure, and the behavioural response of a fish. Brothers & McFarland (1981) found 5 distinct eco-behavioural stages in the early life history of haemulids. Changes in the behavioral response to environmental cues resulted in changes in growth form of the otoliths in the labyrinth system. Similarly, many studies have found growth discontinuities in otoliths that are correlated with habitat shifts and behavioural change; the most common example is checks at settlement in reef fish (Victor 1983a, Pitcher 1988, Fowler 1989). Studies of the sensory and somatic development of reef fish both within and between species, together with measures of performance, will be important aids in the interpretation of the processes which influence the numbers reaching the adult population.

Chapter 4

Variability in age and size at settlement in a reef fish: how much is there and what can it tell us about the process of settlement?

4.1 Synopsis

Age, size and body mass at settlement for the reef fish, *Upeneus tragula*, were examined among five sampling stations across the northern Great Barrier Reef, over time periods ranging from days to three years. Increments on the sagittal otoliths were validated as daily using tetracycline treatment. Fish were caught in their late pelagic stage, brought into the laboratory and those that metamorphosed and settled to the bottom of the tank overnight were used in the study. Twenty-five samples of newly settled fish (n = 6 - 27, mean 14, N = 345) were compared. Larval durations ranged from 25 to 37d, and standard length (SL) showed similar levels of variability, ranging from 19 to 31 mm SL at settlement. Wet weight ranged from 0.09 to 0.61 g. Significant differences in age, length and weight at settlement were found at all spatial and temporal scales. For instance, no consistent pattern in settlement characteristics was found among stations across the northern shelf, with one sample of settled fish often being significantly different in attributes from another sample from the same station a month or two later. Growth rate averaged over the whole larval period ranged from 0.55 to 1.0 mm / d. Fish that settled larger tended to have lower growth rates than smaller fish. Patterns of increment widths throughout the larval phase were remarkably consistent among samples. However, the relationship between otolith length and fish length was found to vary significantly among samples collected within two months from four locations. Results suggest the cautious use of back-calculation of larval size from otolith increment widths. The present concepts of larval growth and settlement in reef fish are re-evaluated. As yet there is no conclusive evidence for delayed settlement in reef fish.

4.2 INTRODUCTION

The size, age and developmental status of fish at settlement may play an important role in determining the subsequent dynamics of reef fish populations. Variation in the duration of the larval period may lead to decoupling of the spawning and recruitment cycles (Victor 1986 b). On the other hand, variability in the age and body size of newly settling fish may influence their survival and role in the reef fish community. Despite the importance of settlement as the link between the larval and demersal life phases, it is only recently that attention has turned to behavioural and physiological events that occur at this transition, and the strong developmental link between events within the plankton and on the reef (e.g. Breitburg 1989, 1991, Champalbert *et al.* 1992 a, b, McCormick & Shand in press, Chapter 3).

Information is now available on the larval duration and size at settlement of over four hundred species of tropical reef fish (e.g. Brothers *et al.* 1983, Thresher & Brothers 1985, 1989, Victor 1986c, Wellington & Victor 1989). Levels of variability within a species range widely and appear to be related to the number of specimens collected (e.g. Thresher & Brothers 1989). Unfortunately, sample sizes are typically small and collected from one site, and as a consequence little is known of the levels of variation. Still fewer studies have examined the relationship between age, size and body mass at settlement over time or across a number of spatial scales. Such information on natural levels of variability in growth over the larval period may suggest processes which determine mortality within the larval phase and the scales at which they operate.

Two hypotheses have been proposed for the mechanisms behind the variability found in size and age of reef fish species. Chambers & Leggett (1987), in a laboratory study of the flounder *Pseudopleuronectes americanus*, proposed that differences in settlement characteristics at metamorphosis were a product of differential larval growth rates. These reflect the interaction of the individual's developmental physiology with exogenous factors (food, temperature). Alternatively, Victor (1986b) reconstructed larval growth rates and fish sizes from the patterns of otolith increment deposition and suggested that the bluehead wrasse *Thalassoma bifasciatum* was capable of delaying settlement.

This latter study has been complemented by the common field and laboratory observation of selectivity in the diel timing of settlement, and choice in the settlement microhabitat (e.g. Marliave 1977, Eckert 1985, Sweatman 1985b, Roberts & Ormond 1987, Booth 1992). For invertebrates, delays in settlement in the absence of settlement cues are commonplace (e.g. Richmond 1985). Together this evidence has resulted in the concept that fish larvae reach a developmental threshold or level of "competence" after which they can be induced to settle if given the appropriate cue. If cues are not forthcoming then settlement can be delayed. In some recent studies this has become an assumption rather than a qualified suggestion (e.g. Cowen 1991, Kaufman *et al.* 1992, Markle *et al.* 1992). The magnitude and duration of this period of choice ("competence") has been inferred from changes in the deposition of microstructural increments in the fishes' otoliths. As this study will show, the lack of understanding of the link between the physiology of the fish and the deposition of otolith increments suggests that inferences from growth increments should be used cautiously and may lead to a spurious idea of settlement dynamics. This chapter examines the evidence for both hypotheses for a particular reef fish species and re-evaluates our current understanding of what happens at settlement.

This study examines the levels of variability in the age, size and body mass at settlement for the freckled goatfish, *Upeneus tragula* (Family: Mullidae). Comparisons are made on a number of spatio-temporal scales: among sampling stations across the northern GBR over one summer (1989/90); among samples collected over a three year period (1989 - 1991) at one inter-reefal station and one station on the backreef of Lizard Island (1988 - 1990). The formation of daily otolith increments is validated and used to construct growth histories of individual fish. Increment widths in the otolith are compared among samples to suggest when in the larval phase the growth trajectories of the cohorts diverged. Information presented suggests that the settlement event is more complex than previously assumed and that the dynamics of it are easily misrepresented.

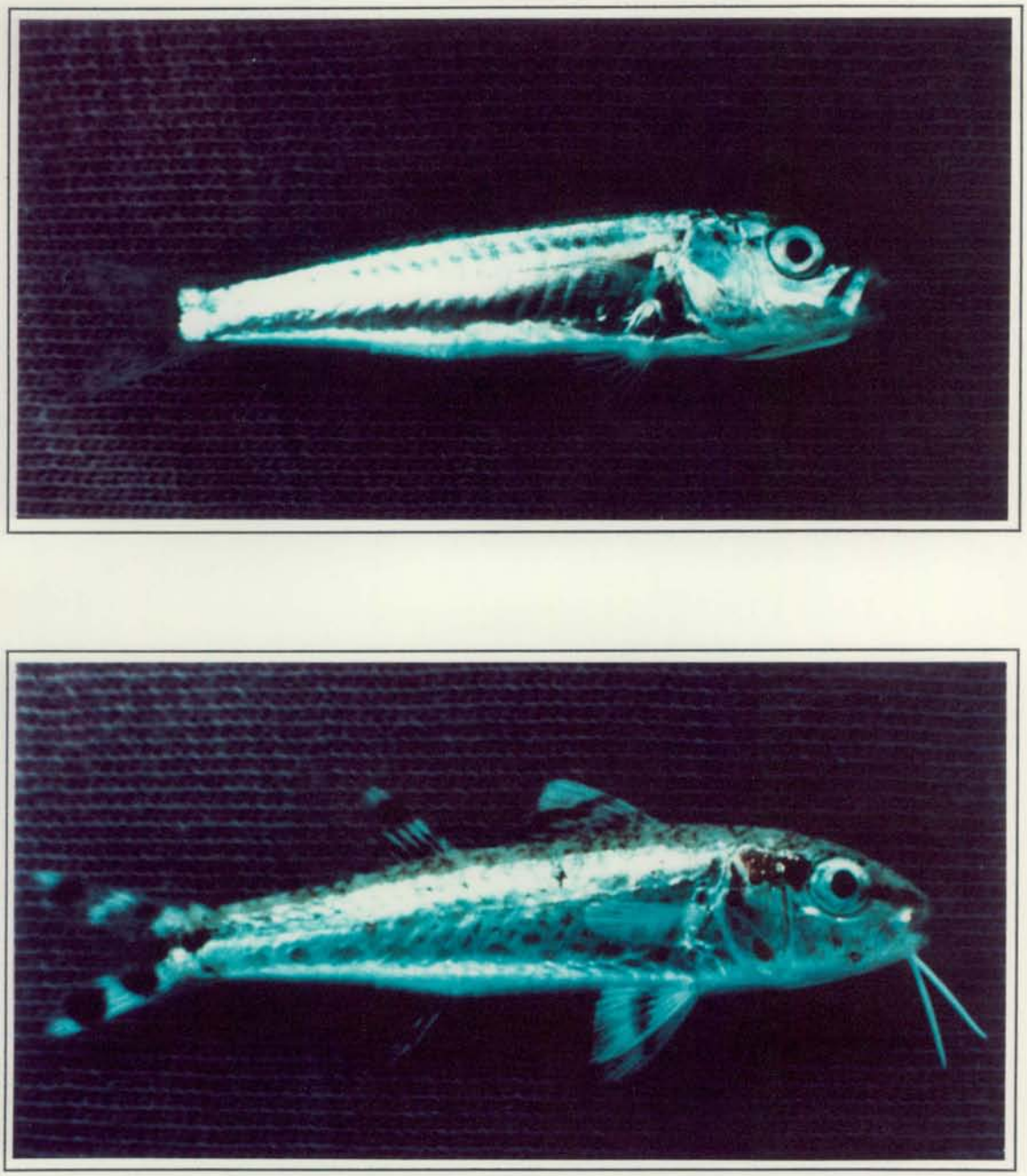

Figure 4.1. *Upeneus tragula*. (Top) Pelagic stage fish just prior to settlement (29.7 mmSL). (Bottom) Newly metamorphosed fish on the day of settlement (29.8 mm SL). (Note: not printed to scale).

4.3 MATERIALS AND METHODS

4.3.1 Sampling

Samples were collected in conjunction with the study of the spatio-temporal distribution patterns of mullids on the northern GBR (Chapter 2). Small plastic rafts (1 x 1 m) were used to aggregate the pelagic stage mullids. Fish were then collected with a plankton-mesh purse seine (14 x 2 m, 0.5 mm mesh) (Kingsford & Choat 1985). Once collected, a subsample of 50 to 1000 fish was transferred to holding tanks of aerated seawater with a dip net, and brought back live to the Lizard Island Research station. Overnight some of the pelagic mullids changed pigmentation and settled to the bottom of the holding tanks. The silver pelagic colouration changes to a mottled cream and fin and body stripes appear (Fig. 4.1). This study assumes that these fish were competent to settle when captured and would have settled that night if they had not been captured. This assumption is addressed in the discussion. These newly settled fish were killed by cold shock, weighed, measured and their otoliths removed.

Upeneus tragula that were competent to settle were collected in this way over four summers (Nov. - Jan.) from Dec. 1988 to Dec. 1991. Three spatio-temporal comparisons of the age and size at settlement were made: (a) across the northern Great Barrier Reef (9 samples 1989/90); (b) one inter-reefal station over three summers (Eagle station, 16 samples from 1989/90 to 1991/92); (c) one station on the edge of the Lizard Island fringing reef (four samples from 1988 to 1990). Sample locations and dates are given in Table 4.1.

Table 4.1. Location, dates (d-m-yr) of samples collected for the comparison of age and size at settlement in *Upeneus tragula*. Number of newly settled fish within a sample is given.

Comparison	Date, *n*
Cross-shelf	Turtle: 19-11-89, 27; 16-1-90, 17. Nymph: 20-11-89, 20; 30-12-89, 23. Eagle: 12-11-89, 6; 28-12-89, 22. Lizard Is.: 30-11-89, 23; 4-1-90, 17. MLC: 21-11-89, 6.
Eagle Station	12-11-89, 6; 28-12-89, 22; 21-1-90, 20; 10-11-90, 10; 5-12-90, 11; 14-12-90, 10; 8-1-91, 10; 10-1-91, 10; 9-11-91, 12; 13-11-91, 10; 14-11-91, 11; 18-11-91, 10; 3-12-91, 11; 8-12-91, 10; 11-12-91, 10; 16-12-91, 10.
Lizard Island Station	16-12-88, 9; 23-1-89, 20; 30-11-89, 23; 4-1-90, 17.

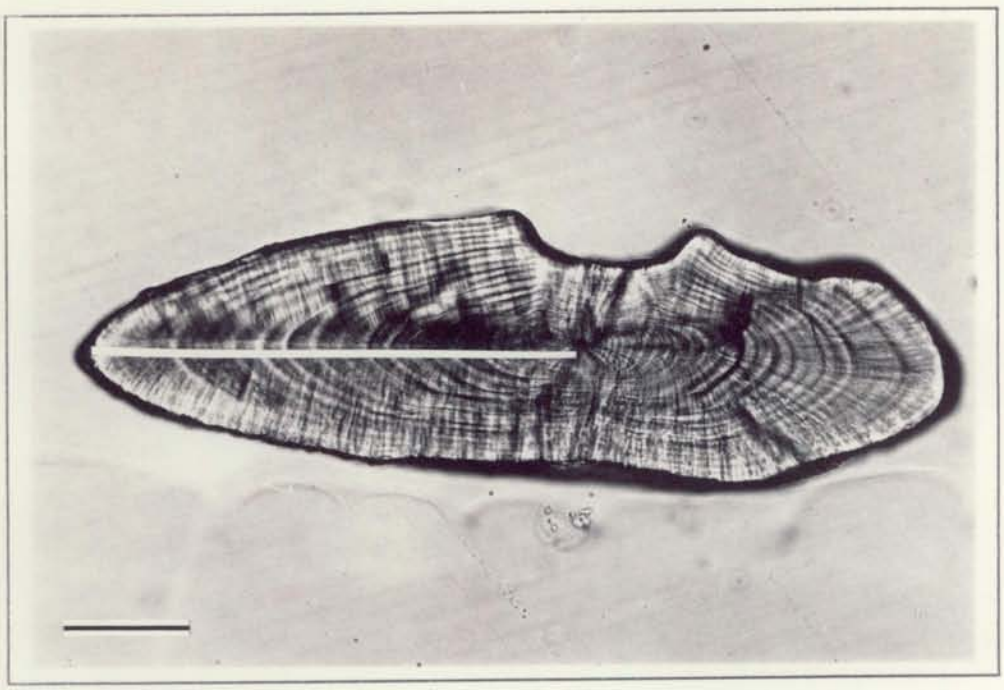

Figure 4.2. Transverse section of a sagitta of newly settled *Upeneus tragula* showing the path along which increment widths were measured (scale: 100 μm).

Five stations across the northern GBR lagoon were sampled once per month for 3 months from Nov. 1989 to Jan. 1990. Station locations were: 9 km N-W of the Turtle Group; 4 km south of Nymph Island; 5 km north of Eagle Island; Lizard Island backreef; midway between Lizard Island and Carter Reef (MLC) (Fig. 2.2). At each station two sites were established; one 500 m each side of a stationary mother boat. At each site, 4 rafts were tethered, with 30 m lines linking the rafts to one-another and to a single temporary mooring. Rafts were left for 4 h and then sampled with the purse-seine. Unfortunately, for some station by month combinations no newly settled fish were obtained, either because no fish were aggregated or none of the fish collected settled the night after capture. Consequently, two samples per station are used in the cross shelf comparison of settlement size and age (except for the station midway between Lizard and Carter reef for which only one was obtained).

Sixteen samples of fish were collected at the Eagle station, in approximately 23 m of water. Four samples were aged for the Lizard Is. station where rafts were moored in 10 - 23 m water depth at the reef edge.

Mullids, unlike the commonly studied damselfishes, seldom settle to isolate patch reefs, or other manipulable substrata. Because of this it is not possible to obtain a sample of fish from the field that, with any certainty, have only just settled. Furthermore, it is not possible to collect a sample of fish that look as though they have recently settled and examine the otoliths for settlement marks since *Upeneus tragula* do not reliably lay down settlement marks in their otoliths (personal obs.).

4.3.2 Ageing

Validation of daily increments

To validate the formation of daily otolith increments pelagic *Upeneus tragula* were immersed in a solution of tetracycline hydrochloride in seawater (250 mg / l) for 12 hours in the dark. This placed a mark on the otolith which was fluorescent under ultraviolet light, and visible under transmitted light as a check. Fish were kept in tanks and fed *Artemia* sp. nauplii and finely chopped prawn for 23 days and nights, then re-immersed in tetracycline hydrochloride for 12 hrs, and kept in tanks for a further approximately 20 days. Otoliths were sectioned as detailed below and the number of increments between the two tetracycline-induced checks was compared to the number of days kept after immersion. The observed number of increments after the induced check mark did not differ from the expected number, with counts ranging from 21.7 to 23 increments (mean of

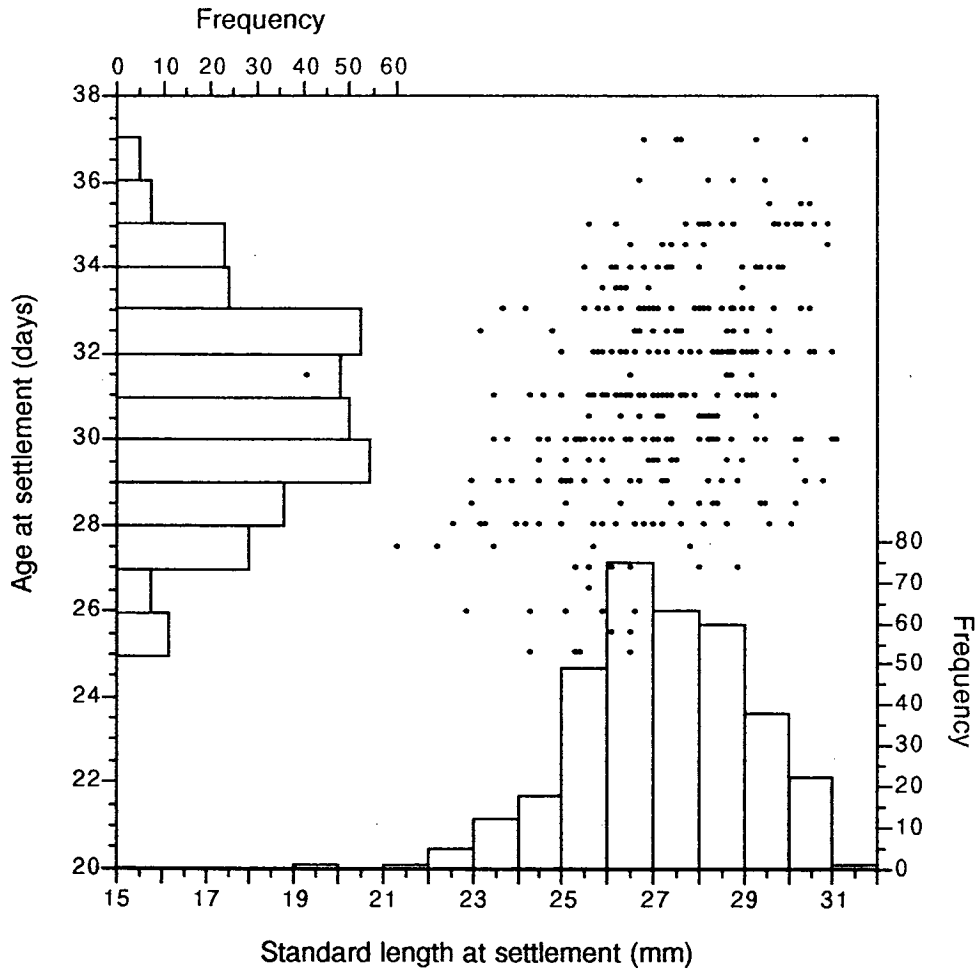

Figure 4.3. Relationship of size (mm SL) to age (d) for newly settled *Upeneus tragula* from the northern GBR. Equation: Age = 0.51(SL) + 17.27, $r^2 = 0.152$, $p < 0.0001$. Overall age and size frequency distributions are also given ($n = 345$).

3 readings per otolith) (mean 22.7, s = 0.35, n = 12). This confirmed the daily formation of increments. To calculate age at settlement this study assumed that increment deposition started at hatching.

Otolith preparation

Transverse sections of the sagittal otoliths were used for ageing. Otoliths were measured along the primary axis and at the widest point perpendicular to that axis using a calibrated ocular micrometer. These were then heated for five minutes on a hot plate, mounted in a spurs-resin block and ground using wet carborundum paper (P600, P1200) and lapping film (9, 3 micron) to produce a transverse section through the nucleus. Otolith increments were read under immersion oil at 1000 x magnification. Sections were read twice and if the counts differed by more than one increment were read a third time. Otoliths with a reading error of greater than 10% were excluded from analysis. Mean increment counts were used in analyses.

For the otoliths of five fish from each of three samples, increment widths from the nucleus to the outer margin were measured on a calibrated video analysis system linked to a compound microscope. The paths of the increment measurements, and increment counts are given in Figure 4.2. Slopes of the 3 samples were compared with analysis of covariance (ANCOVA).

The relationship between size and otolith length for each of two samples from four cross shelf sampling locations was examined with ANCOVA. This tested the null hypothesis that the population regressions were coincident. A Tukey's test compared the intercepts among the eight samples to determine the nature of the significant difference found with ANCOVA (Zar 1984).

4.4 RESULTS

4.4.1 Overall size, age and weight distributions

The age at settlement for *Upeneus tragula* from the northern Great Barrier Reef ranged from 25 to 37 d, with a mode of 29 d and a mean of 31.1 d (s = 2.5 d). The distribution was normal with a broad peak between 29 and 33 d (Fig. 4.3). The standard length at settlement showed a similar pattern, although skewed, with a number of individuals being distinctly smaller. Settlement size ranged from 19 to 31 mm SL, with a mode of 26 and mean 27.2 mm SL (s = 1.9 d). Overall there was slightly less variation in the size than in the age at settlement (CV: 0.069, 0.079 respectively). There was a

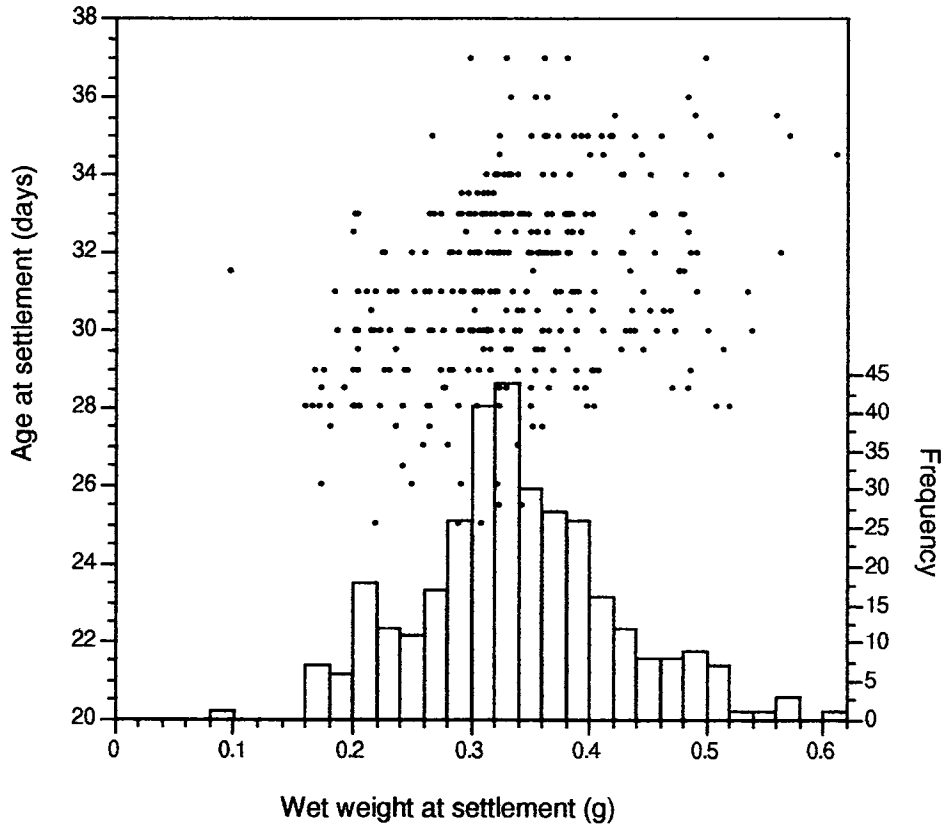

Figure 4.4. Relationship between wet weight (g) and age (d) of newly settled *Upeneus tragula* from the northern GBR. Equation: Age = 9.51(wt) + 2.8, $r^2 = 0.11$, $p < 0.0001$. A weight frequency histogram is given.

poor relationship between length and age at settlement (r^2 = 0.152, p < 0.0001, n = 345; Fig. 4.3).

Table 4.2. Comparison of the mean age (days) and size (mm SL) at settlement and larval growth rate (mm / d) of *Upeneus tragula* among stations across the northern GBR, between Dec. 1988 and Dec 1991. (a) Comparison of mean ages of nine samples from five stations across the northern shelf; (b) Comparison of 16 samples at the Eagle station; (c) Comparison of 4 samples from the Lizard Island station.

Comparison	Source	DF	MS	p
(a) *Cross shelf*				
Age	Sample	8	47.083	0.0001
	Error	152	3.107	
Size	Sample	8	30.567	0.0001
	Error	152	2.789	
Growth	Sample	8	0.0132	0.0022
	Error	152	0.0041	
(b) *Eagle Station*				
Age	Sample	15	19.557	0.0001
	Error	167	3.095	
Size	Sample	15	8.778	0.0001
	Error	167	1.554	
Growth	Sample	15	0.0190	0.0001
	Error	167	0.0030	
(c) *Lizard Is. Station*				
Age	Sample	3	61.176	0.0001
	Error	65	4.7129	
Size	Sample	3	35.481	0.0001
	Error	65	3.418	
Growth	Sample	3	0.0071	0.4112
	Error	65	0.0073	

Wet weight at settlement ranged from 0.09 to 0.61 g, with a mean of 0.34 g (s = 0.08 g) and mode 0.33 g (Fig. 4.4). Not surprisingly, weight showed higher levels of variation compared to age or size (CV: 0.25). Weight was poorly related to age of the fish (r^2 = 0.11, Fig. 4.4). However, weight did show a strong relationship with standard length of the fish, although there was a surprising amount of variation around the exponential

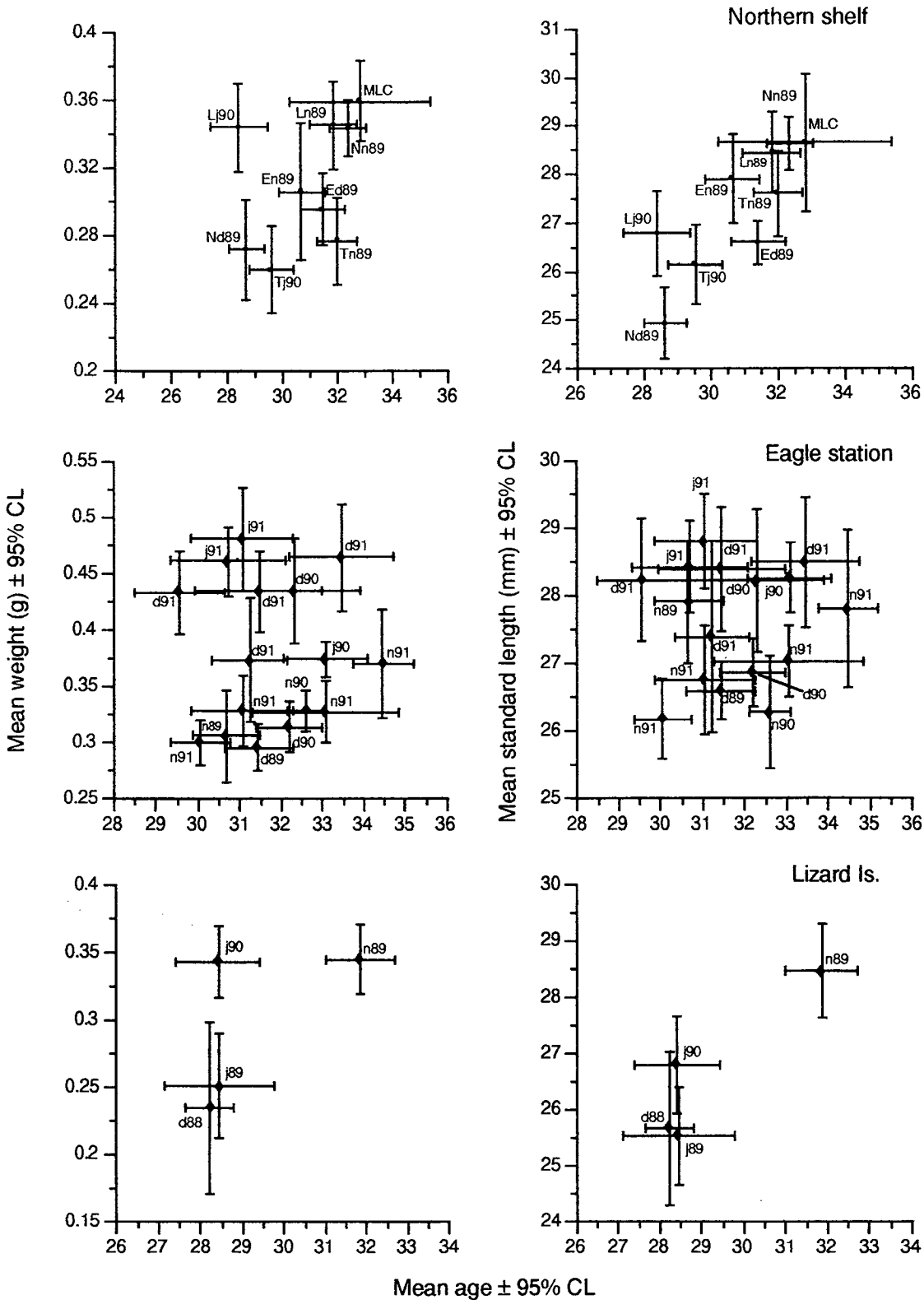

Figure 4.5. Growth characteristics of samples of newly settled *Upeneus tragula* (*n* = 6 - 27). Three comparisons are made among: 5 stations across the northern GBR; 16 samples over three summers from the Eagle sampling station; 4 samples over 2 summers from the Lizard Island backreef station. Notations: capitals for stations (T, Turtle; N, Nymph; E, Eagle; L, Lizard; MLC, midway between Lizard Is. and Carter Rf), lower case letters for months (n, Nov; d, Dec; j, Jan).

relationship (r^2 = 0.653, p < 0.0001, n = 338; Equation: wt = $0.015e^{0.112(SL)}$).

4.4.2 Spatio-temporal variation in size, age and weight

When samples of newly settled *Upeneus tragula* collected over a range of spatial and temporal scales were compared, significant differences were found in the size, age and weight of the fish at all scales (Table 4.2, Fig. 4.5). Stations across the northern shelf showed no consistent pattern in size, weight or age at settlement, with one sample of settled fish at a station often being significantly different in attributes than another sample a month or two later (Fig. 4.5). At the Nymph station fish in the December sample settled on average 4 days earlier, 13% smaller and 21% lighter than fish in the November sample.

Table 4.3. Breakdown of the total variation in age (d), size (mm SL) and weight (g) of *Upeneus tragula* at settlement and their estimated larval growth rates (mm / d) for eight samples collected in two month (Nov., Dec. 1991) at the Eagle station. Significance levels (p in brackets) and mean values for each month are given.

Source of variation	Mean age	Age	Mean size	Size	Mean wt	Weight	Mean growth	Growth
Month	Nov: 32.2	0%	26.9	19.7%	0.33	48.6%	0.781	20.7%
	Dec: 31.4	(0.591)	28.1	(0.035)	0.42	(0.008)	0.835	(0.063)
Sample(month)		49.3%		5.8%		8.9%		15.0%
		(< 0.0001)		(0.086)		(0.004)		(0.006)
Residual		50.7%		74.5%		42.5%		64.3%

Similar levels of variation were found at the Eagle station for 16 samples, over 3 summers (Fig. 4.5). Here significant differences in age, size or weight were often found between consecutive samples from the same month, at times only separated by a single day (Table 4.2 b). For instance, the mean larval duration of a sample of *Upeneus tragula* collected on 13 th Nov. 1991 was 33 d compared to 30 d for a sample collected the following day. Levels of variability in the three variables were formally compared for the eight samples collected from the Eagle station during Nov. and Dec. 1991 (4 each month) (Table 4.3). Age at settlement was not influenced by the month of sampling, with variation in settlement age being split between variation among samples within a month, and variation among fish within a sample (49.3, 50.7% of the total variation respectively). On the other hand,

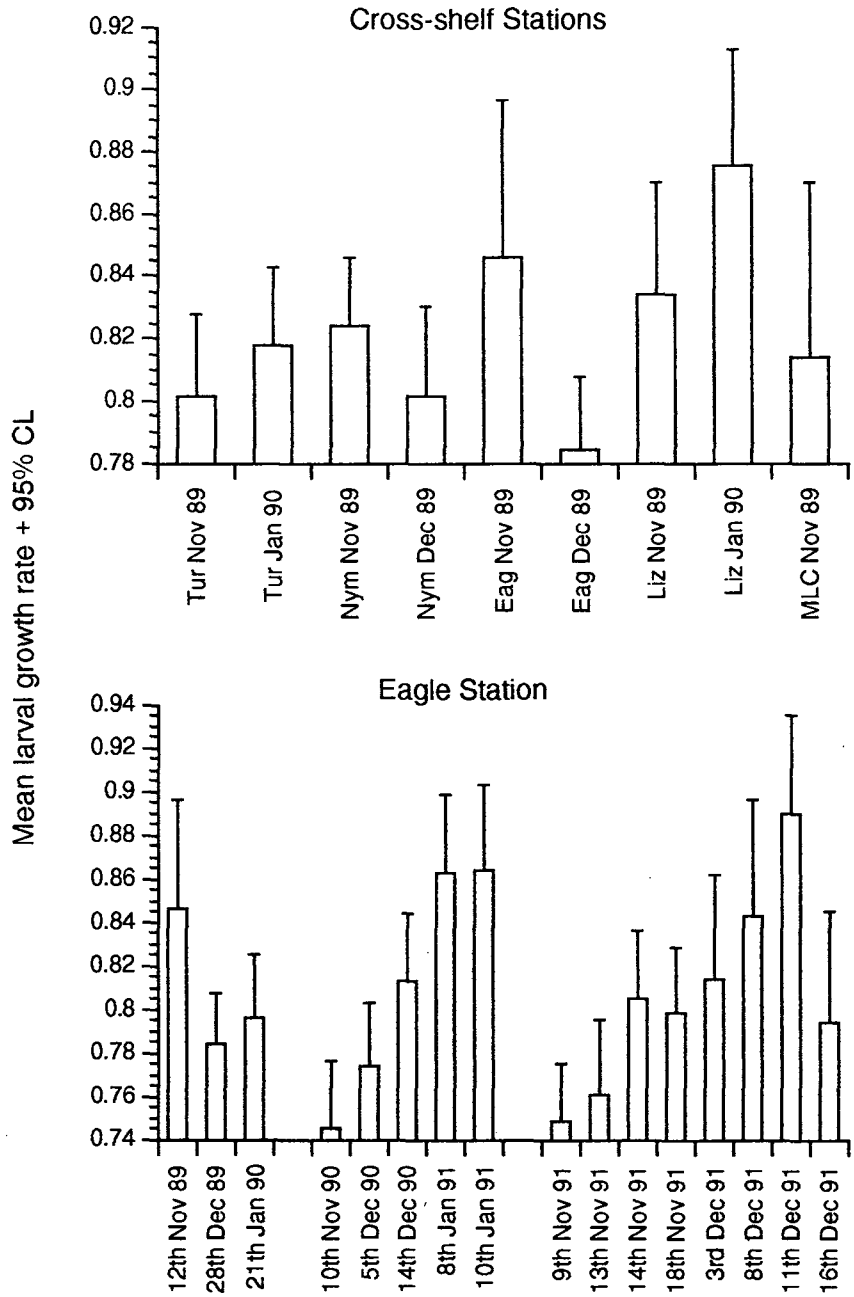

Figure 4.6. Comparison of the mean growth rates (mm / d) of *Upeneus tragula* averaged over the whole larval period among 9 samples collected across the northern GBR, and among 16 samples from the Eagle station (Tur, Turtle; Nym, Nymph; Eag, Eagle; Liz, Lizard; MLC, midway between Lizard Is. and Carter Reef). Error bars are 95% confidence limits.

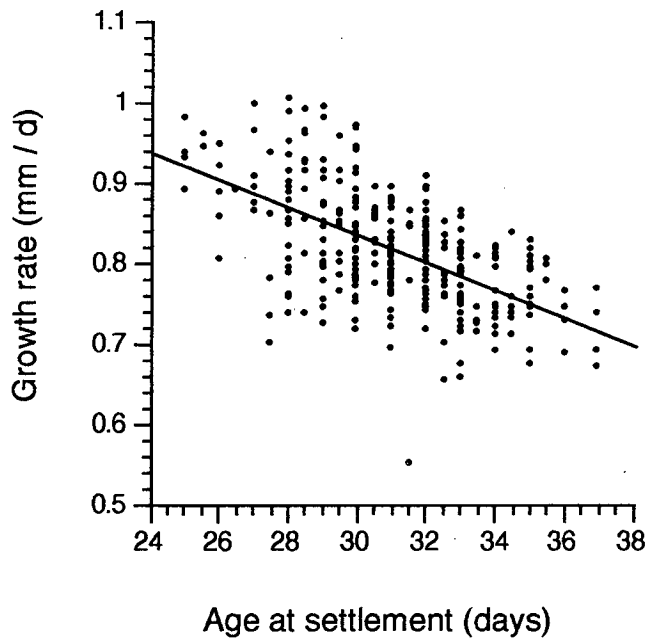

Figure 4.7. Relationship between average growth rate during the larval phse and age at settlement for *Upeneus tragula*. Equation: GR = -0.0167(Age) + 1.33, r^2= 0.34, $p < 0.0001$, $n = 345$.

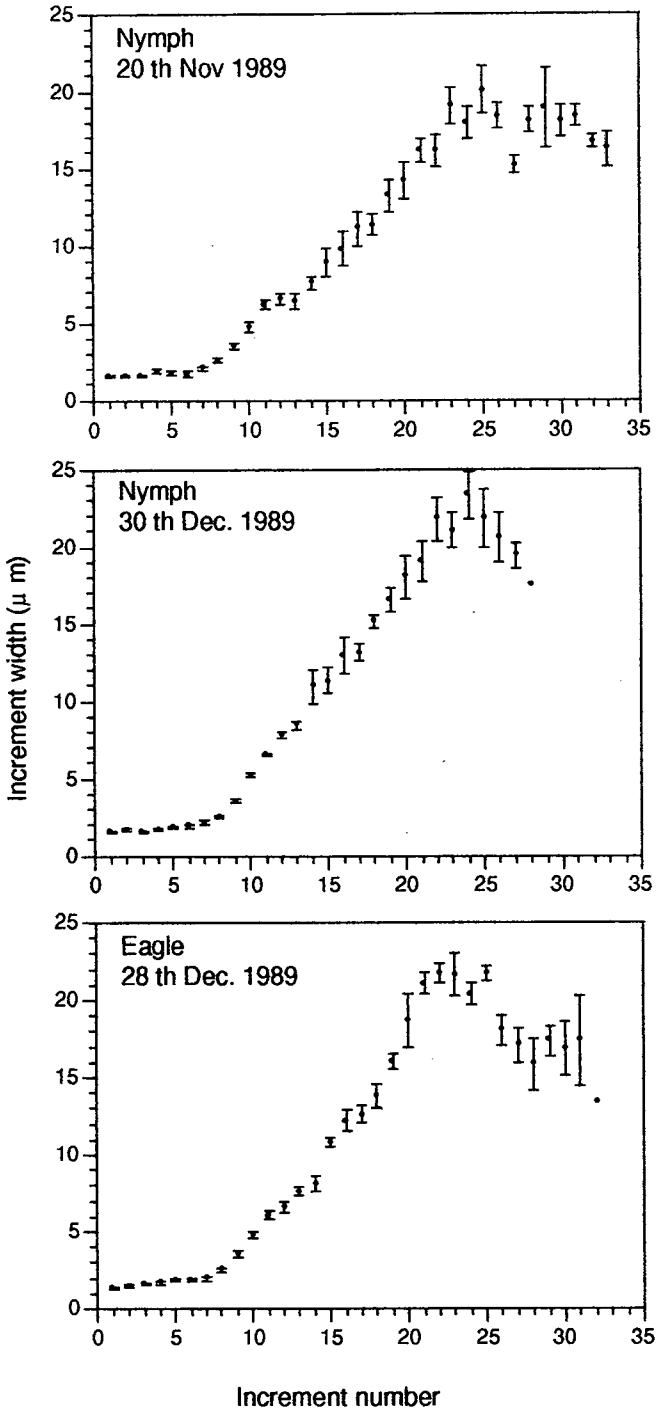

Figure 4.8. Increment widths from cross sections of the sagitta from three samples of newly settled *Upeneus tragula*; two samples from the Nymph station and one from the Eagle station. Error bars are standard errors (n = 5 fish).

the month of collection was an important determinant in the standard length and weight of fish at settlement, accounting for significantly greater amount of the total variation than differences among samples within a month (19.7 vs 5.8%, SL; 48.6 vs 8.9% wt). Both size and weight were greater in the December samples (Table 4.3). *Upeneus tragula* collected from the Lizard Island backreef further emphasises the notion of significant variability occurring between samples at a single location (Table 4.2 c, Fig. 4.5).

4.4.3 Growth rates

A rough approximation of the average larval growth rate to settlement was calculated by dividing the difference between the settlement and hatching size by the age at settlement. Leis & Rennis (1983) have an illustration of a 2.6 mm mullid larvae that is well formed and has absorbed its yolk which is most likely *Upeneus tragula*. Hatching size is therefore estimated at 2 mm SL. Growth rates estimated in this way ranged from 0.55 mm to 1 mm / d (n = 345 fish). Larval growth rates differed among samples across the northern shelf (Table 4.2a, Fig. 4.6) and among 16 samples from the Eagle station (Table 4.2b, Fig. 4.6). Growth rates showed a significant increase over the sampling dates within two of the three summers for which data was collected (with the exception of the last sample from the 1991/92 summer). Growth rates did not differ among four samples from the Lizard station (Table 4.2c). A breakdown of the variation in growth rates among eight samples collected over two month at the Eagle station found that 21% of the variation in growth rate was explained by differences between sampling months (Table 4.3). A further 15% of the variation was explained by differences among samples within a month, while 64% of the variation was attributable to difference in growth rates among individual fish. Interestingly, fish that settled larger tended to have lower growth rates averaged over the pelagic phase than fish that were smaller at settlement (Fig. 4.7).

4.4.4 Increment spacing and the otolith / fish size relationship

Increment widths on the sagittal otoliths were examined to provide *suggestions* as to the origin and nature of the differences in the average larval growth rates among samples. An examination of the increment widths of two samples from Nymph station one month apart (Nov and Dec 1989), plus a third randomly selected from the other possible 23 samples (Eagle station, Dec. 1989), illustrates two points (Fig. 4.8). Firstly, the pattern of increment spacings is remarkably consistent among all three samples up

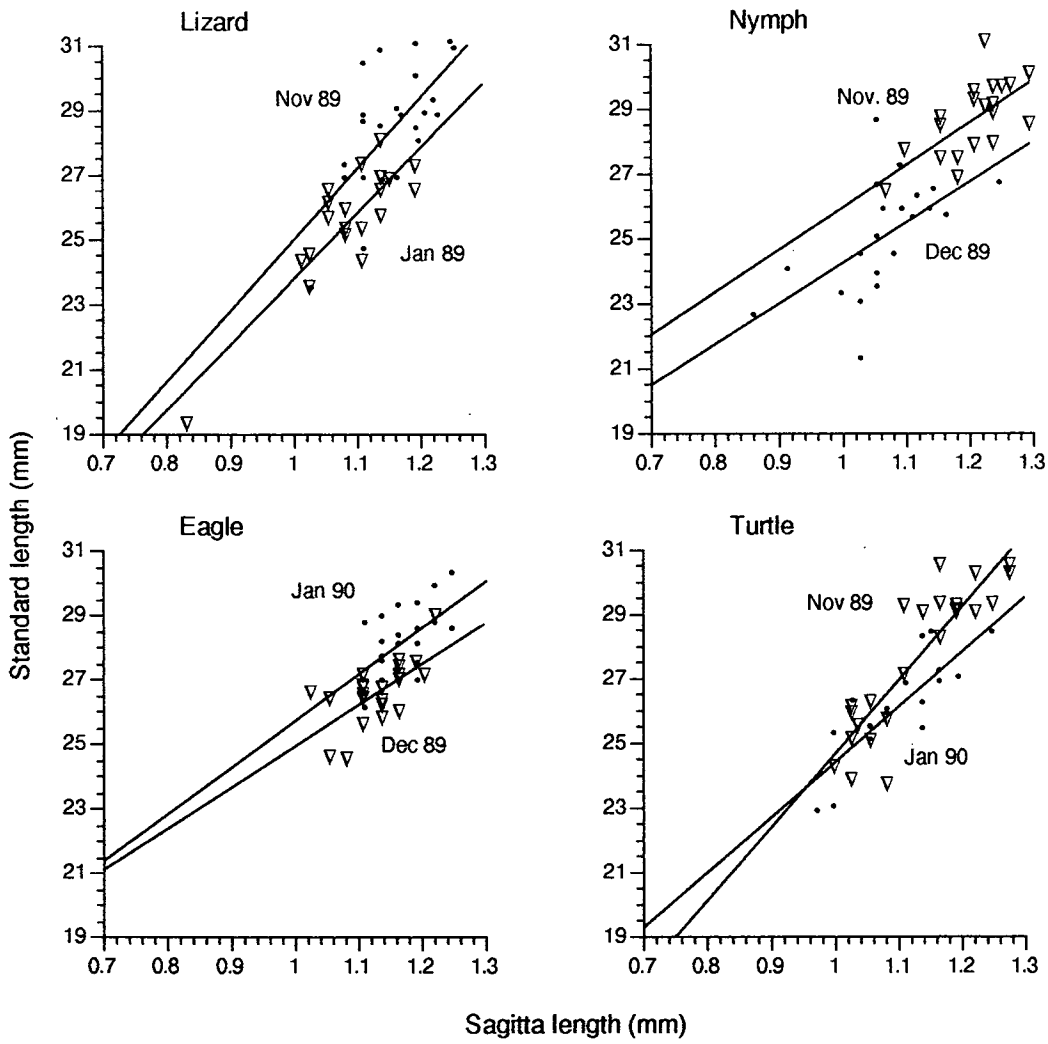

Figure 4.9. Relationship between standard length and sagittal otolith length for eight samples of newly settled *Upeneus tragula* from four stations. Latter of the two sampling dates per graph plotted as triangles.

until increment 23 - 24. After this point there is an abrupt end to the previously steady increase in otolith increment width. Secondly, the rate of increase in increment width is significantly higher, reaching an overall greater width, in the December samples than in fish caught in November (d.f. 1, 176, $p < 0.0001$). Examination of otoliths from field-settled *Upeneus tragula* show no other rapid narrowing of increments.

Table 4.4. Comparison of the relationship between sagittal otolith length (OL) and standard length (SL) among eight samples of newly settled *Upeneus tragula* from four stations across the northern GBR. (a) ANCOVA summary table for tests of homogeneity of slopes and elevations. (b) Regression equations and coefficients calculated individually for each of eight samples. All regressions are significant at $p < 0.001$. Statistical groupings from pairwise comparisons of adjusted mean elevations are given.

a. Source	D.F.	MS	F value	*p*
Slope	7, 154	2.020	1.68	0.1177
Elevation	7, 161	8.141	6.58	0.0001

b. Grouping	Station, date	Regression equation	*n*	r^2
[	Lizard, Nov. 89	SL = 21.99*OL + 3.00	23	0.44
[	Turtle, Nov. 89	SL = 22.86*OL + 1.77	27	0.78
[	Eagle, Jan. 90	SL = 14.64*OL + 11.08	20	0.35
[	Nymph, Nov. 89	SL = 12.90*OL + 13.04	20	0.43
[	Turtle, Jan. 90	SL = 17.14*OL + 7.26	17	0.67
[	Eagle, Dec. 89	SL = 12.71*OL + 12.20	22	0.45
[	Lizard, Jan. 89	SL = 20.35*OL + 3.40	20	0.75
[	Nymph, Dec. 89	SL = 12.49*OL + 11.75	21	0.34

The overall relationship between otolith length and standard length was found to significantly differ among eight samples collected from four stations across the northern GBR (Fig. 4.9, Table 4.4 a). No difference was found in the slopes of the regression lines among samples (Table 4.4 a). This may be due to the low numbers of fish used to define each regression (17 to 27). However, the intercepts of the regressions did differ among

samples, with significant differences occurring at all stations over time (Table 4.4 b).

4.5 DISCUSSION

The growth characteristics of the freckled goatfish at settlement were found to vary significantly across sampling stations separated by kilometres, and at specific stations over time periods ranging from days to years. Similar levels of variability were found between days, years and among individuals within a single sample. The levels of variability identified in the age, size and mass of the settlers and their inter-relationship, aid in identifying the factors that may potentially influence the settlement event. Furthermore it stresses the importance of the pelagic life history stage in shaping the demography of juvenile reef populations.

The levels of variability found in the size at settlement for *Upeneus tragula*, expressed as the coefficient of variation, span all other published accounts (0.028 - 0.082, mean 0.051) (e.g. Robertson *et al.* 1988: damselfish 0.062, Wellington & Victor 1989: damselfish 0.04 - 0.07, Victor 1991: wrasse 0.055 - 0.063). Similarly, levels of variation among samples in the age at settlement range widely (CV: 0.02 - 0.1, mean 0.06). Thresher & Brothers (1989) found a strong relationship for Pomacentrids between the number of fish aged from a species and variability in its larval duration. However, for *Upeneus tragula* the differences in the levels of variability shown cannot be attributed to sampling artefacts, since no such relationship exists among samples of different sizes ($F_{1,23} = 0.227$, $p = 0.23$).

The sample sizes used in this study are similar to those typically used to quantify the larval durations and size at settlement for a species (6 - 27) (e.g. Wellington & Victor 1989, mean $n = 7.2$ fish). The high and significant variability shown to occur among samples cautions against drawing biogeographic or regional conclusions on the range of larval durations from small sample sizes, collected at one time and location. Differences have been found before in the larval duration of reef fishes between areas or over time. Thresher *et al.* (1989) examined the larval duration of *Pomacentrus coelestis* from 5 locations in western Pacific. Despite a broad overlap, the mean age varied significantly among locations, ranging from 18.6 d to 20.7 d. Wellington & Victor (1989) noted a significant difference between the larval duration of the damselfish,

Stegastes partitus, in St Croix (mean = 28.8 d, *n* = 14) and Panamá (mean = 36.5 d, *n* = 206). They also found that *S. flavilatus* in Panamá had a much shorter larval life (mean = 27 d, *n* = 5) than individuals in Ecuador (mean = 35.4, *n* = 5). Differences found in the present study at the Eagle station in both size and age at settlement of *Upeneus tragula* between samples a day apart, suggests that the geographic differences found in these and other studies (Victor 1986c, Thresher & Brothers 1989, Thorrold & Milicich 1990) may simply be the product of small scale temporal variation at a single site. Information on the levels of variation at the lower spatial and temporal scales (e.g. among and within sites and months) is necessary to validly interpret results at higher levels (e.g. among geographic locations).

Recently, Wellington and Victor (1992) statistically examined the differences in the larval duration of the labrid, *Thalassoma lucasanum*, over three geographic locations in the eastern Pacific. At each location two samples of juveniles were collected, with individual samples at each location separated by a time frame of up to 7.5 years. No differences were found in the larval durations between the two samples for any of the three locations. This result may have been due to the low replication within a location. However, the wide spread of the collections through time, and the consistency of the result among locations suggests that, for *T. lucasanum* at least, small scale temporal and spatial variation within a location may contribute little to the overall variation in larval durations among geographic locations. The high variation in the larval durations among samples of *Upeneus tragula* found in the present study (49% of the total variation), suggest this result is not universal and more attention is required to small scale spatial and temporal variation. Sampling schemes should incorporate all potential levels of variation in a hierarchical framework, as is commonly recommended by standard texts on ecological sampling (e.g. Green 1979, Underwood 1981). A better handle on levels of intraspecific variability in larval durations will enhance our ability to examine hypotheses concerning species integrity and genetic dispersal. Dispersal characteristics of a species will be linked to the individuals in the upper tail of the distribution of larval durations rather than the mean duration.

Getting realistic estimates of levels of variability in larval durations and size at settlement that are comparable to published accounts can be difficult given the range of methodologies used. Methodologies can be divided into the six categories listed in Table 4.5. *All* methods have inherent

biases and problems that are likely to affect the mean and variance estimates. Many of these will be species dependent. A summary of some of the potential effects have been listed in Table 4.5.

The most commonly used technique is the back-calculation of settlement dates (and sometimes sizes) from a sample of the juvenile population (e.g. Thresher *et al.* 1986). Checks in the otolith, that are assumed to be due to the physiological and behavioural disruption associated with metamorphosis and settlement, are used as labels from which larval rings can be counted. Check identification is often a subjective process as otoliths are typically not as clear as those shown in publications, being marred by ghost rings and sub-daily rings. To compound matters, the form of the settlement check differs greatly among species (e.g. Victor 1983a, 1986 b, c, Pitcher 1988, Fowler 1989, Lou In press), and sometimes it is not until an experienced reader has examined a number of otoliths from the same species that its characteristics and approximate location become clear (pers. obs., Lou pers. com.). Such problems may not bias the mean settlement age but may bias its associated variance estimate by the exclusion or inclusion of unusually large or small presettlement counts. Size- or age- related mortality during the early days on the reef may further bias the sample. Given all these potential problems it is optimistic that Pitcher (1988) found that back-calculation from otolith increments reconstructed the known patterns of settlement of two pomacentrids at Lizard island with considerable precision. Unfortunately, inconsistent relationships between otolith size and fish size, as shown here and in other studies (e.g. Thorrold & Milicich 1990), complicate the back-calculation of fish size at settlement or larval growth rates. The differences in the relationship found between months from the same location and between locations are of major concern and shed doubt on the conclusions of studies that have used a single pooled relationship to back-calculate fish sizes. In concert, these problems caution the use of back-calculation until the biases of the methodology (precision and accuracy) have been properly examined for the species of interest.

The methodology used in the present study, where a whole school or random subsample of pelagic stage fish are brought back to the laboratory and held overnight while some settle, is the least biased method of obtaining settlement ages and sizes. This method yields all fish that are potentially capable of settling to the reef. As with all the methods of

Table 4.5. Likely and potential biases of the mean and variance estimates of age and size at settlement of the main collection methods used (√, unbiased estimate; ↑, overestimate; ↓, underestimate; ?, unknown effect).Referenced examples: 1, Brothers & McFarland 1981, 2 Victor 1984, 3 Pitcher 1988; 4, Hunt von Herbing & Hunte 1991, 5 Thresher & Brothers 1985, 6 Wellington & Victor 1992, 7 Victor In press, 8 Brothers *et al.* 1983, 9 Robertson *et al.* 1988, 10 Policansky 1983, 11 Fukuhara 1986, 12 Chambers & Legget 1987, 13 Thorrold & Milicich 1990, 14 this study, 15 Avise & Shapiro 1986, 16 Wellington & Victor 1989.

	Methodology	Age		Size		Potential problems
		Mean	Variance	Mean	Variance	
1	Back-calculation of settlement from juveniles[1,2,3,4,5,6,7]	√?	↓	?	?	Subjectivity of identifying settlement checks; Inconsistent relationship of otolith size/fish size; size or age dependent mortality prior to collection.
2	Reef collections from patch reefs on day of settlement[8,9]	√?	↓?	√?	↓?	Migration/mortality prior to collection may be size or age dependent.
3	Larval rearing[10,11,12]	?	↑	↓↑	↑	Tank artefacts, establishment of dominance hierarchies.
4	Pelagic sample[13]	↓	↑	↓	↑	Include fish that are not yet competent to settle.
5	Pelagic sample settled in laboratory[14]	√?	√?	√?	√?	If delay in settlement possible then may lead to inducement of premature settlement in fish that would not have settled in the field.
6	Smallest reef fish, Largest from plankton[15,16]	↓ ↑	↓ ↓	↓ ↑	↓ ↓	Under represent large settlers from reef, and small settlers from the plankton.

collecting fish to estimate larval durations, experimental studies are required to examine the biases of the method. If further studies find that the larvae of some species are stimulated to settle earlier than they would under field conditions, then this method still yields an estimate of fish that are competent to settle. Under these circumstances, the larval durations may be slight underestimates and have higher associated variance estimates, that will lead to conservative statistical tests among samples. The increasingly widespread use of night lights to attract larval fish, and the wide array of taxa attracted, lend this technique to general use (e.g. Thorrold & Milicich 1990).

The ecological consequences of variability in size when a fish comes back onto the reef are yet to be examined. In juvenile fish, and for larval fish in tanks, size has been found to correlate with social dominance and the acquisition of food (e.g. Coates 1980, Folkvord 1991). This extends to mate choice, retention and reproductive success (number of spawnings and fecundity) in adults (e.g. Hoffman 1985, De Martini 1988). For newly settling fish, length related characteristics may greatly affect which individuals are lost to predation during the early life on the reef. A potentially more important measure of fish quality may be its weight or bulk. Predictably this will vary more than length being exponentially related to length during the rapid growth phase of the larval fish (in this study overall CV's, 0.069 SL v.s. 0.25 wt). The current study found that the heaviest settling fish was seven times the weight of the lightest. Body mass also has the potential to influence success (measured as survival or reproductive output) independent of length, since length only explained 65% of the variability in weight. After settlement these length- and weight- related pressures have the potential to enhance initially large differences in the larval growth rate (0.55 - 1 mm / d), and extend their influence well into the established reef population.

Few data exist on the processes which govern the levels of variability in larval growth rates and the age and size at settlement. The influence of density dependent factors on larval growth rates is unknown for reef fish. If growth rate is density dependent then a strong regulatory mechanism exists to stabilise the numbers of potential recruits (Houde 1989a). Feeding history and water temperature during the pelagic stages have been suggested as potentially important determinants of larval growth rates and body attributes at settlement (Wellington & Victor 1992). In this

study, growth rates averaged over the whole larval period tended to be higher in the samples collected from the Eagle station in Dec. and Jan. compared to those from Nov., for the summers of 1990/91 and 1991/92. Although there was no significant difference in growth rates among the three Nov. / Dec. 1989 samples for which increment patterns were measured, the slope of the increment widths between 7 and 24 d was significantly lower in the Nov. sample than for the two Dec. samples. If otolith increment spacing is related to somatic growth, then this suggests that the growth rate prior to day 23 - 24 was higher in the December. These differences in growth rate and increment spacing may be due to the temperature difference. Temperatures on the back-calculated spawning dates (using temperature data in Chapter 7) differed between November and December samples by 2 °C. Differences of this magnitude have been found to be sufficient to influence the growth potential of reared fish larvae (Buckley 1982, Rana 1990). However, the influence of temperature and food availability are difficult to tease apart due to the effects of temperature on phytoplankton productivity. Samples from the Eagle station collected over two months suggest that the processes of growth (in size) and differentiation (to metamorphosis) of the larvae are not necessarily influenced by the same factors. Experiments which examine the interaction of temperature and food availability on the growth parameters of fish larvae are required to elucidate the mechanisms driving the observed variability.

Recently it has become an assumption that once a reef fish has reached a state of physiological "competence" it can delay settlement until it comes across an appropriate settlement habitat (e.g. Cowen 1991). Patterns of increment deposition on the otoliths have been interpreted as records of development and growth (e.g. Victor 1986b, Kingsford & Milicich 1987). In the present study, the shapes of the increment curves were very similar among samples, even though fish differed by as much as eight days in their settlement ages. Day 8, when the rate of increase in increment width abruptly increases, probably represents first exogenous feeding. Interestingly, in all three samples the increase in increment width abruptly stops and begins to decrease at about day 24. The suggestion that the somatic growth rate decreases after this point until settlement is supported by the strong negative relationship between growth rates averaged over the whole larval period and fish age at settlement. Such a trend could be interpreted, as it has in the past (Cowen 1991), as evidence for delayed

settlement, with day 24 representing the attainment of a "competence" threshold.

I suggest that this is a misinterpretation of the increment pattern for two reasons. Firstly, the youngest fish to settle from all of the fish aged was 25 days old. If physiological "competence" acted as a threshold then the settlement-age frequency distribution (Fig. 4.3) should be skewed towards the lower ages, with a considerable number of fish (or at least some!) settling on day 23 and 24. The age frequency curve is, however, normal with a median of 31 d. The same argument against a competence threshold can be made when competence is defined as the attainment of a specific size, as determined by the smallest individual found to settle (e.g. Victor 1986b, Wellington & Victor 1992). Secondly, given the levels of variability in the biological and physical environment of the fish (e.g. temperature, food availability and quality etc.), and given the variable response of the fish to these conditions (growth rates, ages, weights etc.), then the consistency of the shapes of the increment width curves suggests an endogenously controlled process, largely divorced from exogenous influences. Higher variability after day 24 suggests that development may be more greatly influenced by exogenous factors in the late pelagic stage. Thus for *Upeneus tragula* at least, the shape of the otolith increment curve may be a blueprint for the development pattern of the species over the localities sampled. The scale to which it fits (i.e. absolute size of increment widths) may, however, be exogenously controlled. This does not preclude the possibility that *U. tragula* may be able to delay settlement, but simply means that presently there is no evidence for it.

The concept of "competence" involves the development of the sensory system to a point where it can function competitively in the new reef environment, or it can undergo the rapid changes sometimes associated with metamorphosis (e.g. McCormick & Shand In press). The development of the sensory systems, be they gustatory, visual or acoustic, have been found to be largely endogenously controlled, with the pattern of their development only being influenced by exogenous forces in periods of stress (e.g. starvation) (Chapter 3). Thus the response of the endogenous controller to internal physiology will place the lower boundary on the timing of metamorphosis. Active choice of the settlement microhabitat is indisputable for most reef fish (e.g. Eckert 1985). The important question is how large is the window of choice and what determines its upper bound. Is

it restricted to a short period after the fish first enters the reef environment, as has been suggested from studies of flounder (e.g. Chambers & Leggett 1987)? In October 1989 a strictly reef associated fish (*Parupeneus multifasciatus*) with reef coloration and juvenile size (≈ 65 mm SL) was observed feeding on plankton around 1 x 1m fish aggregation raft, 5 km from Osprey Reef in the Coral Sea over 1000 m of water (B. A. Kerrigan, JCU, pers. com.). This observation suggests that maybe the upper temporal bound to settlement is dependent on appropriate settlement cues for some species and not inextricably linked to metamorphosis.

In summary, this study has shown that at times large and significant differences can exist in the size, age and growth characteristics of reef fishes at settlement over short time scales and within and among locations. These need to be taken into account if the link between the pelagic life history and demersal reef population is to be understood. The growth attributes of newly settling fish may not be all the same as is commonly assumed. At present the causes of this variability can only be speculated upon. Evidence suggests that the pattern of larval development may be endogenously controlled while the magnitude of that growth is controlled by exogenous factors such as nutritional and temperature regimes. Further information is required on the mechanism underlying metamorphosis and settlement if we are to understand the limits to genetic dispersal in open populations, and the determinants of the quality of newly recruiting reef fish.

Chapter 5

Quality of the reef fish, *Upeneus tragula*, at settlement

5.1 Synopsis

The quality at settlement of the goatfish, *Upeneus tragula*, was examined from 10 samples collected over two summers (1990/91, 1991/92), at one location near Lizard Island on the northern Great Barrier Reef. Nine commonly used measures of fish condition were employed: standard length (SL); body depth at the pectoral fin; total lipid content; carbohydrate content; protein content; water content; growth rate; burst swimming speed, and Fulton's condition factor ($K = wt / SL^3$). High and significant levels of variability were found in all measures of condition over the 10 samples. Correlations of measures of condition with fish length were very poor. Specifically, burst speed and stored energy levels (lipids, carbohydrates) had particularly low correlations with fish length ($r \leq 0.2$). An experiment determined that burst speed of a fish at settlement was not related to its feeding history within the larval stage. Feeding history only accounted for 6 % of the total variation in burst speeds. Most of the variability in measures of condition were due to differences among individuals. The poor correlations between all measures of fish quality (with exception of morphological measures) suggests no single measure gives an adequate description of a fishes' survival potential. Furthermore, this study cautions against the direct transfer of size-based theories of success (i.e. "bigger is better"), to the variability within a specific developmental state.

5.2 INTRODUCTION

Recent reviews of larval ecology stress the importance of events that occur during the early larval stage in influencing the quality or health of the larvae, and ultimately which individuals survive to recruit into the juvenile population (e.g. Miller *et al.* 1988, Pepin 1989, 1991, Srivastava & Brown 1991). These reviews stress the multiplicative benefits of being larger than average throughout the larval period, where an advantage in size flows on to advantages in sensory and somatic development, capture of food, and escape from predation (Bailey & Batty 1984, Eaton & DiDomenico 1986, Miller *et al.* 1988, Bailey & Houde 1989, Miller *et al.* 1992). Although many researchers have looked at condition of fish larvae, most have examined it over a wide size range, spanning a range of developmental states (e.g. Yin & Blaxter 1987, Fuiman 1989, Margulies 1989). When such an array of developmental states, ranging from naive to competent, are incorporated into the same analysis, strong positive relationships with measures of success (e.g. growth rate, mortality rates, biochemical condition etc) are to be expected (e.g. Margulies 1989). To date, researchers have been interested in the shape of the relationship between size and aspects of condition, and have ignored the associated variability.

It is of interest to know the levels of variability in traits that measure potential success or condition at a particular developmental stage, and whether the same hypotheses regarding survival capabilities hold at this smaller scale. Small differences in the growth rate and quality during the larval stage, when the processes of differentiation and growth are fastest, can determine the strength of a recruitment event (Miller *et al.* 1988, Beyer 1989, Houde 1989a). Furthermore, on an individual level, these differences in quality may be accentuated by post-recruitment processes and influence which fish reach maturity.

For reef fish, the transition from pelagic larva to demersal juvenile can represent an important numerical filter (Blaxter 1988), during which time larval condition may play a crucial role. Recent studies identify settlement as a period of major physiological (Pfeiler & Luna 1984) and developmental remodelling (McCormick & Shand In press). Some fish do not feed during this metamorphosis (e.g. Pfeiler 1986), which may take hours to months (Pfeiler & Luna 1984, Markle *et al.* 1992), instead having to survive on energy reserves stored during the pelagic life stage. The present

study examines the quality of a newly settled reef fish from ten samples collected over two summers from the same location on the northern GBR. In doing so it highlights the potential importance of the high levels of variability in condition found at this transition.

Many methods have been used to measure aspects of the physiological and structural quality in fishes (Bolger & Connolly 1989). These can be categorised into direct methods (e.g. morphological measures) and derived methods (e.g. residuals from regressions of logged length and lipid levels, Suthers *et al.* 1992). The present study compares nine direct methods of assessing the quality of a reef fish at settlement. Two morphological measures are compared with more intensive biochemical determinations of body composition, growth rates, Fulton's K (condition factor) and burst speed. Here, burst speed is used as a measure of the fishes' ability to survive, being important to both the success of capturing food items (Laurence 1972) and ability to escape predators (Webb 1981). Since the feeding history within the pelagic stages has been shown to affect the growth characteristics of a reef fish at settlement (McCormick & Molony 1992), it is of interest to know whether feeding history also influences the fishes' burst speed when they first encounters the reef population.

Specifically, we use the newly settled stage of the goatfish, *Upeneus tragula*, to approach the following questions. How much variability is there in the quality of a newly settled reef fish measured in various ways? Does food availability during the pelagic stages influence a fish's probability of survival at settlement? How do standard methods of measuring condition (e.g. Fulton's K) compare to more detailed methods? Is there a good, easily measured predictor of quality of this reef fish at settlement? Finally, to what extent can the size-related theories of which factors determine survival in the plankton (i.e. "bigger-is-better", Litvak & Leggett 1992) be transferred to the period of settlement to the reef? In answering these questions I emphasise that fish of a particular species do not start their reef associated life with an equal probability of survival.

5.3 MATERIALS AND METHODS

5.3.1 Sampling

Ten samples of pelagic-stage mullids were collected 5 km north of Eagle Island on the northern GBR, over two summers (1990/91, 1991/92) (Fig. 2.2). Floating plastic rafts (1 x 1 m) were used to aggregate the mullids, that were then caught with a 14 x 2 m plankton-mesh purse seine (0.5 mm mesh). All *Upeneus tragula* were carefully transferred into lidded 30 to 70 l black buckets containing aerated seawater. Fish were then rapidly transported to the Lizard Island Research Station, where the buckets were connected to a flow-through seawater system. Those fish that settled the night after capture were subsequently processed. It was assumed that these individuals were competent to settle when caught and would have settled that night.

5.3.2 Variability in the quality of newly settled fish

All newly settled fish were killed by cold shock. Standard length (SL), body depth at the pectoral fin (BDP) and anal fin, and wet weight were obtained fresh. Fish were preserved in liquid nitrogen for biochemical examination. Total lipid, carbohydrate, protein and water content (mg / g wet wt) were determined for ten fish randomly selected from each sample. Biochemical determinations were duplicated for each fish. Methods are detailed in Chapter 6.

5.3.3 Measurement of burst speed

Burst speeds were measured in a long narrow tank (5 x 20 x 75 cm) which restricted the fishes' movement largely to two-dimensions. The back and sides of the tank were blackened and scale bars were placed on the back of the tank. Water temperature during the burst speed trials was approximately 28 °C. Newly settled fish were placed into the burst speed tank, left for 3 - 5 min to acclimatise then stimulated. This involved tapping the fish with a perspex probe at the base of the tail. After approximately 10 sec, the fish was left to recover for 3 - 5 min and then stimulated again. This was continued until exhaustion or a until a number of good bursts had been recorded. Burst-motion was obvious and three fish that failed to give a burst speed under these conditions were excluded from the analysis. A video camera was used to record the fishes' movements (Panasonic NVN7A, 25

frames / s). The progression of the head was traced and the distance moved between consecutive frames of the video tape was determined (Panasonic video recorder AG7330 linked with Jandel video analysis software), allowing the maximum burst speed to be calculated.

5.3.4 Effect of feeding history on burst speed at settlement

The influence of feeding history on the burst speed of the fish at settlement was examined. Pelagic-stage *Upeneus tragula* were fed five different food quantities until settlement. Pelagic *U. tragula* were caught as detailed above and those of a similar length (20 - 23 mm SL) were randomly placed into ten 60 l aquaria (25 fish / tank), representing two tanks in each of the five feeding treatments. Fish were fed 36 to 48 h old *Artemia* sp. nauplii (Artemia 90 brand). Food was added to each of the tanks four times a day, in concentrations that were calculated to result in the following densities after introduction: (a) Fed *ad libitum* (1600 nauplii / l); (b) 4/5 Fed (1280 nauplii / l); (c) 3/5 Fed (960 nauplii / l); (d) 2/5 Fed (640 nauplii / l); (e) 1/5 Fed (320 nauplii / l).

Fish that metamorphosed and settled overnight in the treatment tanks were removed prior to feeding the following morning. Fish were in the tanks for between 6 and 34 days (mean 15.6d) prior to metamorphosis. Burst speeds were obtained for each settled fish, and their morphology and weights recorded as above.

5.3.5 Comparison of measures of condition

Nine methods of quantifying fish condition were compared: Standard length (SL); body depth at the pectoral fin; total lipid content; carbohydrate content; protein content; water content; growth rate; burst speed, and Fulton's condition factor ($K = wt(kg) / SL(m)^3$). All nine indices were measured for each fish ($n = 100$, except for burst speed where $n = 80$). These were chosen due to their extensive use in the literature. Average growth rate over the larval phase was determined by dividing the length at settlement (minus an estimated hatching length of 2 mm) by the age at settlement. Age was determined by counting microstructural increments on transverse sections of the sagittal otoliths. Increments are formed on a daily basis (Chapter 4). Trends in the nine measures of condition were compared graphically over the ten samples of newly settled *Upeneus tragula*, and the correlations among measures were examined.

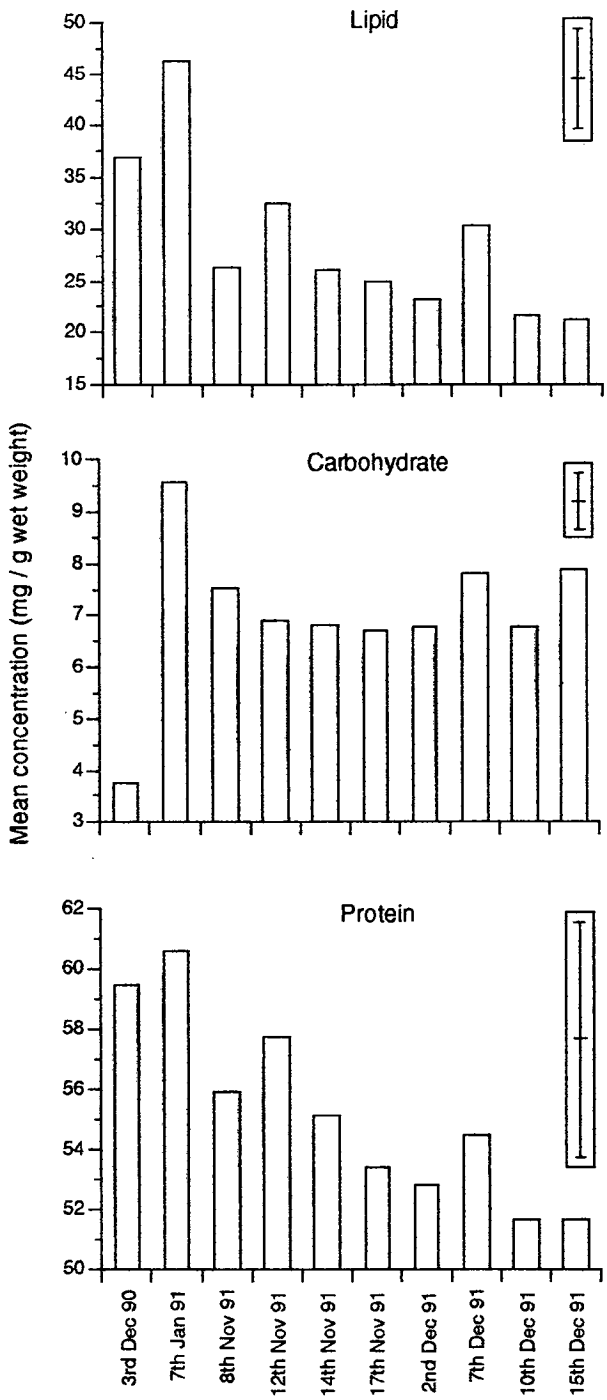

Figure 5.1. Content of total lipid, carbohydrate and protein in newly settled *Upeneus tragula* from ten samples over two summers. Pooled 95% confidence limits are given (n = 10).

5.4 RESULTS

5.4.1 Variability in the quality of newly settled fish

The levels of total lipids, carbohydrates and proteins were found to vary significantly among the ten samples of newly settled *Upeneus tragula* (Table 5.1). Levels of mean lipid and protein concentrations showed close correspondence (Fig. 5.1), with those samples registering high lipid levels also having high protein levels. Mean lipid levels per sample ranged from 21 to 46 mg / g wet weight. Carbohydrates showed no relationship to protein or lipid levels. Although the two samples for the 1990/91 summer were highest in lipids and proteins they exhibited both the highest and lowest levels of carbohydrate (Fig. 5.1).

Table 5.1. One-way ANOVA's comparing the body composition and morphological attributes of newly settled *Upeneus tragula* over ten samples taken over 2 summers (n = 10, except for burst speed where n is variable). * log transformed.

Variable	Source of variation	DF	MS	p	Percent variation
Lipid	Sample	9	622.32	0.0001	52.7
(mg / g)	Residual	90	51.16		47.3
Carbohydrate*	Sample	9	0.111	0.0001	70.6
(mg / g)	Residual	90	0.003		29.4
Protein	Sample	9	98.93	0.0024	17.7
(mg / g)	Residual	90	31.35		82.3
Water*	Sample	9	0.0001	0.4572	1.5
(mg / g)	Residual	90	0.0001		98.5
Standard length	Sample	9	6.87	0.0006	21.0
(mm)	Residual	90	1.88		79.0
Growth rate	Sample	9	0.0204	0.0001	32.0
(mm / d)	Residual	90	0.0036		68.0
Fulton's K	Sample	9	22.50	0.0001	67.0
(g / mm^3)	Residual	90	1.056		33.0
Burst speed	Sample	7	402.09	0.0867	4.8
(cm / s)	Residual	157	220.77		95.2

None of the biochemical constituents measured showed a strong relationship with standard length (Fig. 5.2), with partial correlation

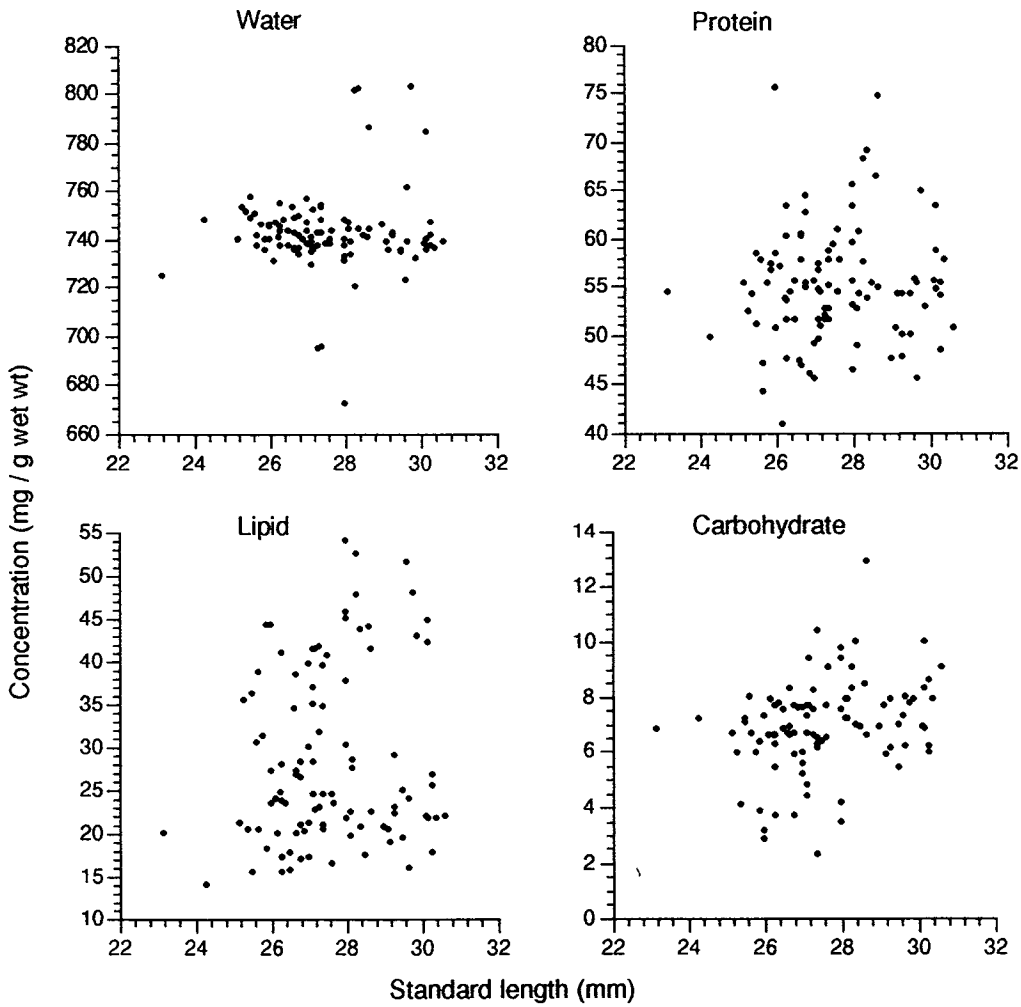

Figure 5.2. Relationship between standard length of newly settled *Upeneus tragula* and their biochemical composition. All are non-significant except carbohydrate ($r = 0.199$, $p < 0.05$).

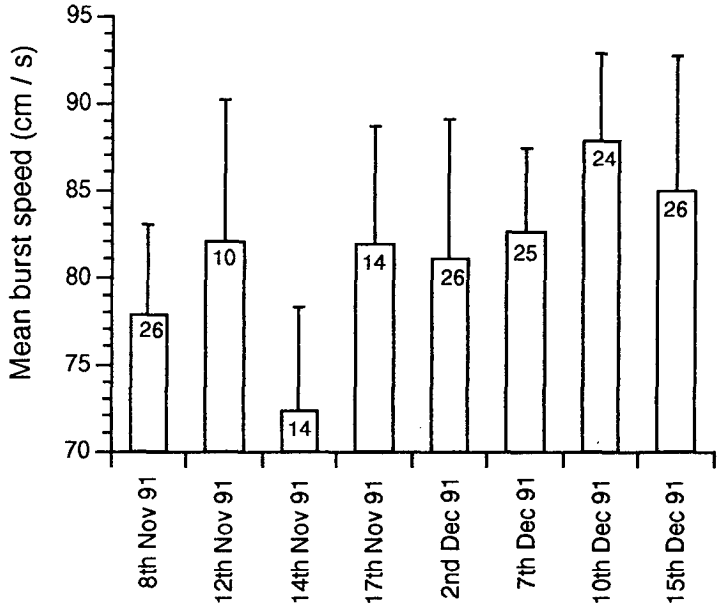

Figure 5.3. Mean burst speed of eight samples of newly settled *Upeneus tragula* collected from one location on the northern GBR. Error bars are 95% confidence limits; *n* is variable and also given.

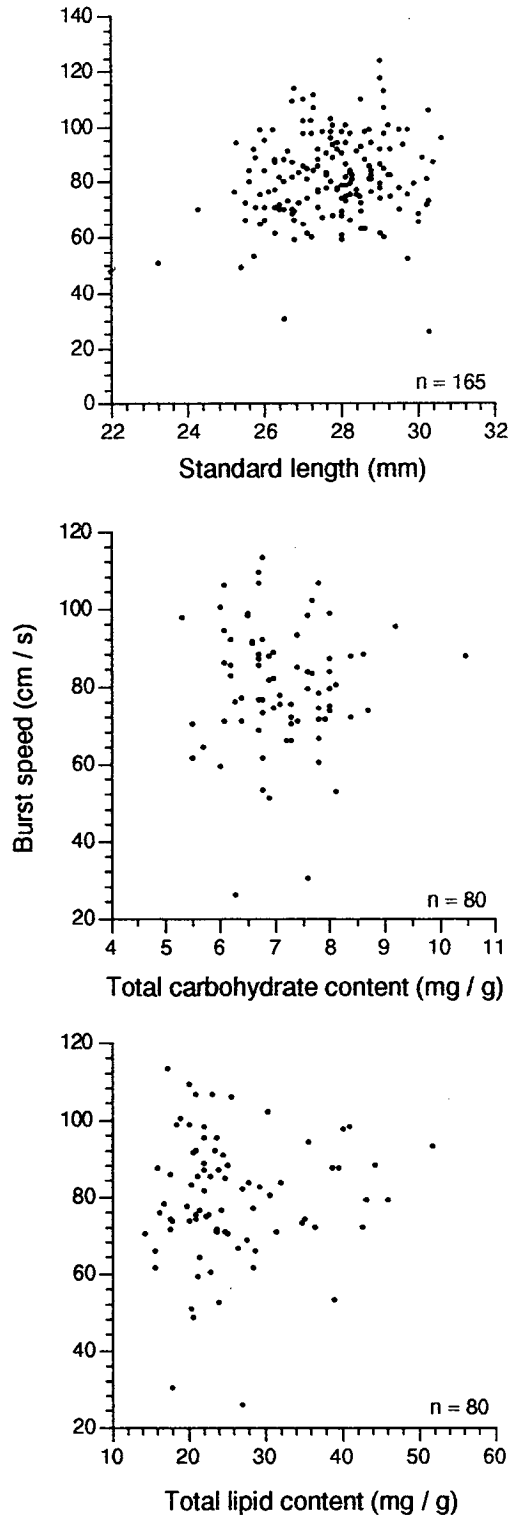

Figure 5.4. Relationship between burst speed and standard length, total carbohydrate and lipid concentrations of newly settled *Upeneus tragula*. All are non-significant (p = 0.22, 0.37, 0.52 respectively).

coefficients (adjusted for variable ages) ranging from 0.03 (protein) to 0.19 (carbohydrate) (Table 5.2). Total carbohydrates showed the highest variability, with a five fold difference between fish exhibiting the lowest and highest levels (2.4 to 13 mg / g wet weight). Lipids also ranged widely among fish (14 to 55 mg / g). Over 50% of the total variation in lipids and carbohydrates was attributable to differences among samples (53 and 71% respectively, Table 5.1). On the other hand, most of the variation in water and protein content was due to differences among individual fish within the samples (98.5, 82.3 %, Table 5.1).

Table 5.2. Partial correlations, adjusted for age of fish, among nine measures of quality of newly settled *U. tragula* . Correlations with body depth at the pectoral fin (BDP) are adjusted for age and SL, except for the correlation between BDP and SL when only age is held constant. Correlations with growth rate are Pearson's correlations. Levels of significance are given (*, $p < 0.05$; **, $p < 0.001$). CHO, total carbohydrate; K, Fulton's condition factor .

	SL	BDP	Lipid	CHO	Protein	Water	Growth	Burst	K
SL	1.000	0.886**	0.089	0.189	0.035	-0.189	0.581**	0.158	0.253*
BDP		1.000	0.165	0.128	-0.314	0.199*	0.488**	0.183*	0.504**
Lipid			1.000	0.200*	0.358**	-0.388**	0.014	0.082	-0.308*
CHO				1.000	0.129	-0.419**	0.060	0.116	0.159
Protein					1.000	-0.365**	-0.006	0.054	-0.457**
Water						1.000	-0.176	-0.069	0.099
Growth							1.000	0.208*	0.144
Burst								1.000	0.281*
K									1.000

Burst speeds did not differ significantly among the eight samples from Nov. and Dec. 1991 ($p = 0.09$, Table 5.1, Fig. 5.3). Ninety-five percent of the variation in burst speed was due to differences among individual fish within samples (Table 5.1). Burst speeds ranged from 25.5 to 123.4 cm / s (8.4 to 42.5 body lengths / s), with a mean of 81.8 cm / s. Interestingly, burst speed showed no relationship with standard length, total carbohydrate or lipid levels of the fish (Fig. 5.4).

5.4.2 Effect of food quantity on burst speed at settlement

The amount of food received during the late pelagic stage had little influence on the burst speed of *Upeneus tragula* at settlement under experimental conditions. Those fish in the 3/5 fed treatment had the highest mean burst speeds, while fishes that received the least food (1/5 fed) had the lowest burst speeds (Fig. 5.5). In this experiment 88 % of the variation in

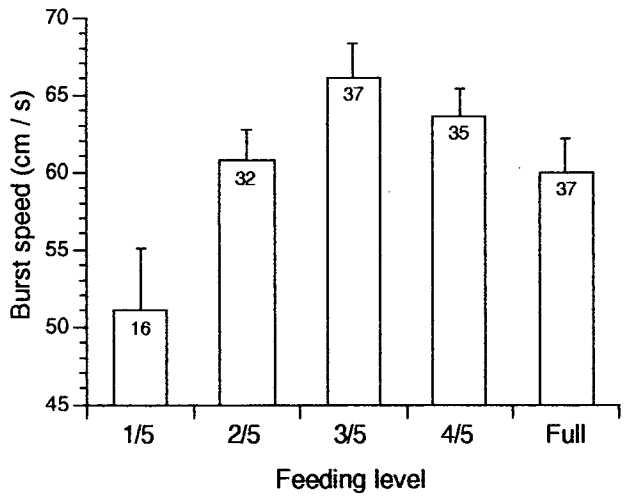

Figure 5.5. The effect of food quantity on the burst speed of *Upeneus tragula* at settlement. Error bars are standard errors. *n* is variable and also given.

burst speed was due to differences among fish within the ten tanks (Table 5.3). Differences among the five feeding levels were not statistically significant (Table 5.3).

Table 5.3. Nested ANOVA examining the effect of five different feeding levels during the late pelagic stage on the burst speed of *Upeneus tragula* at settlement. The proportion of the variation explained by each factor is also given.

Source of variation	DF	MS	*p*	Percent variation
Treatment	4	630.87	0.2596	6.0
Tank(treat.)	5	348.52	0.0644	6.3
Residual	146	163.16		87.7

Time taken to settle was negatively related to food received, with those fish in the 1/5 feeding treatment taking twice as long to settle as fish fed *ad libitum* (24.8 c.f. 11.4 d). However, only 5% of the variation in burst speeds among treatments was explained by the time spent in the tanks prior to settlement ($r^2 = 0.05$, $p = 0.008$, $n = 156$).

5.4.3 Comparison of measures of condition

Overall, there was little correspondence among the trends in the mean values of the nine measures of condition over the ten field samples (Fig. 5.6). However, there were exceptions. Fulton's K exhibited a similar trend over the ten field samples to that of fish growth rate. Body depth at the pectoral fin and total carbohydrate content also showed a strong similarity. Lastly, total lipid and protein content closely mapped one another. Trends in the burst speed among samples bore no relationship to trends in any of the other eight measures of condition (Fig. 5.6).

On the level of individual fish, the correlations among measures were poor, with the exception of relationships between morphological measures (Table 5.2). Notably, growth rate over the whole larval period was significantly correlated with both standard length and body depth at the pectoral fin. Predictably, total lipid, carbohydrate and protein contents were negatively related to water content (Weatherley & Gill 1987). Fulton's condition factor recorded some of the highest correlation coefficients with other measures of condition, and yielded the highest number of significant correlations (5). Burst speed faired the worst with only 2 significant, but

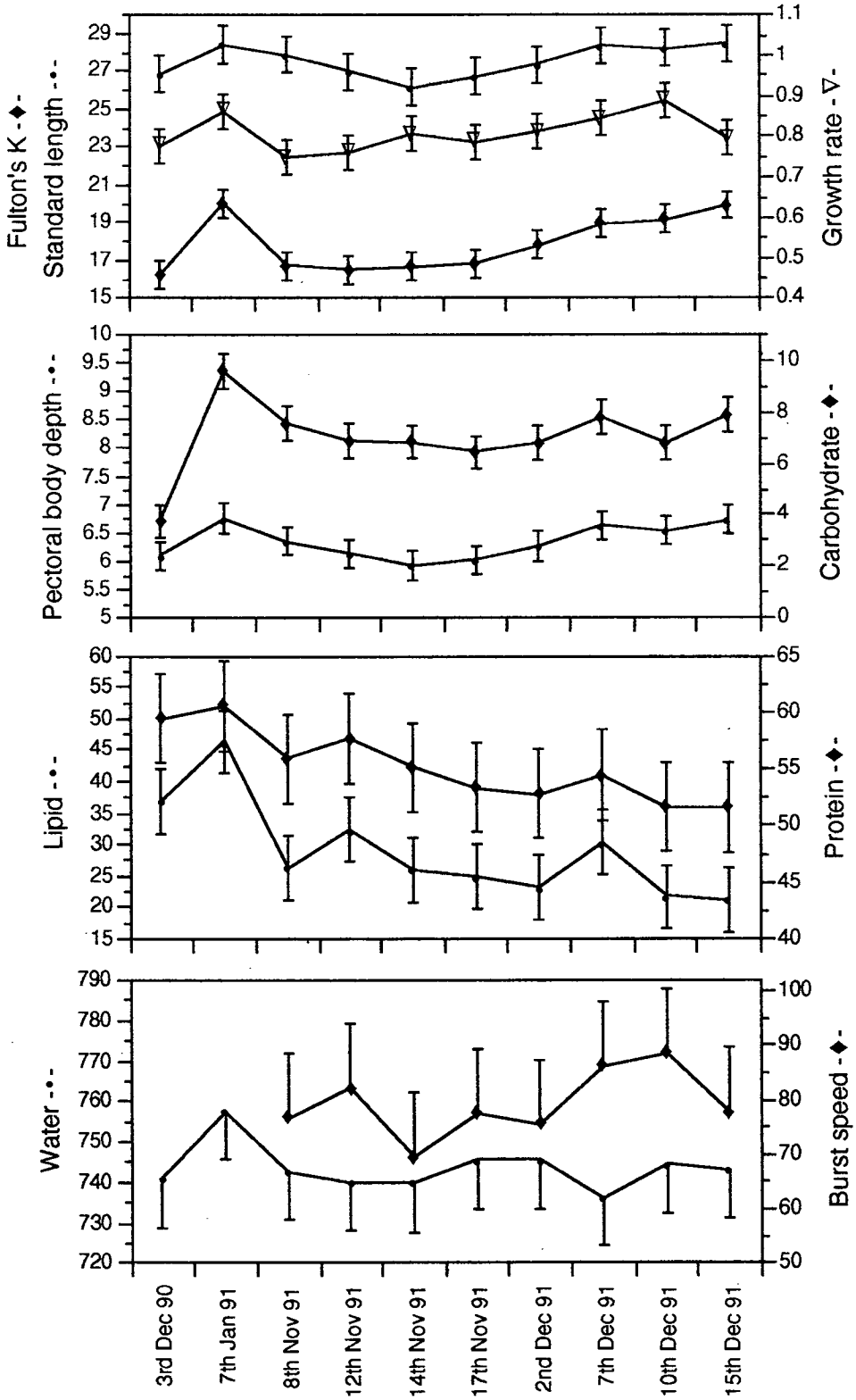

Figure 5.6. Comparison of nine commonly used measures of fish condition in assessing the quality of ten samples of newly settled *Upeneus tragula*. Error bars are 95% confidence limits (n = 10).

poor, correlations (Table 5.2). Stepwise multiple regressions of the five most easily measured indices of condition (i.e. SL, BDP, K, water content and growth rate) on burst speed, lipid, carbohydrate and protein content (on both non-transformed and logged data) were performed but the predictive ability was not increased over that of simple regressions.

5.5 DISCUSSION

This study found high and significant variability in the condition of 10 samples of newly settled *Upeneus tragula* collected from one location on the northern GBR over two summers. Interestingly, the most ecologically meaningful measure of potential survival and success, burst swimming speed, was poorly correlated with standard methods of assessing condition. These conclusions have ramifications for the way we view reef fish replenishment. All reef fish do not start their reef associated existence with equal probabilities of survival and success. Furthermore, the relative differences among fish at this developmental stage are not easily quantified. Size cannot simply be used as a proxy for condition.

The lack of relationship between fish length and burst speed conflicts with published accounts (e.g. Webb & Corolla 1981, Fuiman 1986). For instance, Bailey (1984) found that escape speed of five larval fish species was strongly correlated with fish length, and both length and escape speed closely predicted a fishes' ability to avoid three invertebrate predators. There are a number of possible reasons for this discrepancy. All other examinations of burst swimming speed have encompassed a range of developmental stages, rather than large numbers of fish from a single developmental stage as has been done in the present study. This alone may account for the difference between results. Further, the highest correlations have been attained for early larval stages, spanning hatching through to first feeding (e.g. Webb 1981, Webb & Corolla 1981), when the behavioural response to the burst stimulus (probe, pipette or electric shock) is largely innate. In contrast, fish at settlement, with more fully developed sensory systems, are likely to exhibit a more individualistic response to the burst stimulus. Observations of newly settled *Upeneus tragula*, in both the field and tanks, suggests they are territorial and solitary for the first few days after settlement, suggesting that they are well developed behaviourally. It is likely that the burst response is modified by the behavioural response of the individual fish to the simulated predator, thereby yielding a response not closely associated with fish length or biochemical composition.

Results from the feeding experiment further emphasised the individuality of the burst response. Differences in feeding history within the pelagic life stage only accounted for 6% of the total variation in burst speed of the fish at settlement. This is of particular interest, since McCormick & Molony (1992) experimentally examined the effect of feeding history on the growth attributes of *Upeneus tragula* at settlement and found that fish fed *ad libitum* during their late pelagic stages had better muscle development than less well fed fish. This lends support to the notion that individual behaviour influences the fishes' maximum non-sustainable speed.

The burst speeds recorded in this study were considerably higher than those recorded for similar sized fish of other species, reaching 42.5 BL / s, with a mean of 29.5 BL / s. By far the highest recorded burst speed is for larval stages (3.6 mm total length) of the Asian cyprinid, *Danio rerio*, at 50 - 66 BL / s (Fuiman 1986). However, burst speeds change ontogenetically (Wardle 1977). Evidence to date suggests that, when burst speed is measured as BL / s, it is generally higher in early larval stages and decreases asymptotically with increasing length (e.g. Webb & Corolla 1981, Wardle & He 1988). Wardle & He (1988) summarised the existing literature in a regression relationship predicting maximum speed for body lengths ranging from 13 mm to 2.4 m. This calculation predicts that newly settled *Upeneus tragula* (mean length 27.8 mm) should have a considerably lower maximum swimming speed of about 19 BL / s (i.e. 53 cm / s). The relatively high burst speeds recorded in the present study may be due to the use of field caught fish, rather than reared fish used in most other studies. A comparison of the speeds of fish trialed the day after capture (Fig. 3) with those from the feeding experiment (Fig. 5), shows that rearing in tanks over the latter third of their pelagic existence causes a marked reduction in burst speed (81.8 c.f. 61.5 cm / s respectively). This suggests that published accounts of maximum speeds of reared fish may be substantial underestimates. It is not until other species of a similar developmental stage are caught from the field and trialed that the importance of the speeds attained by *Upeneus tragula* can be assessed.

In a similar way to burst speed, there was high variation in the energy storage components (lipids and carbohydrates) among the newly settled fish. A large proportion of this variability was due to differences among samples (53 and 71% respectively), which stem from the pelagic ecology of the goatfish. Chapter 2 found that the sampling strategy used in

this study targets schools of larval goatfish. Once caught under rafts, fish were brought back to the laboratory where some metamorphosed and settled overnight. These newly settled fish are likely to have similar feeding histories, having experienced the same vagaries of food abundance and quality. If the schools are persistent this will result in the similarity of the biochemical composition found for *Upeneus tragula* within samples. For a reef fish at settlement the amount of energy stored as readily accessible carbohydrates and lipids may be important due to the changes in habitat, feeding mode, and competitive regimes associated with the transition between pelagic and reef environments. The change to juvenile foraging patterns may take hours (in planktivores) to days (in demersal forages, McCormick unpublished data), and during this time of lower feeding efficiency, stored energy may be used in competitive interactions or predator avoidance.

The low correlations among the nine measures of condition (with the exception of SL and BDP) suggest that, within this developmental stage, there is no single measure of condition that will comprehensively describe a fish's ability to survive and compete in its environment. Each measure will address specific questions regarding the fish's condition. For instance, length, with its strong correlations with other morphological variables such as body depth and weight, may be useful in predicting the outcome of competitive interactions. On the other hand, lipid and carbohydrate content may be important in assessing which fish would best survive limited or unpredictable food resources. Theilacker (1978) noted that while morphological measures may reflect the nutritional condition of larval jack mackerel, *Trachurus symmetricus*, no one criterion accurately identified larval condition on an individual basis. Neilson *et al.* (1986), in a laboratory study of the Atlantic cod *Gadus morhua*, found that neither Fulton's condition factor nor relative body height was positively correlated with the performance of larvae in avoiding a simulated predator. I support the view of Theilacker (1978) and Neilson *et al.* (1986) that the rapid physiological and morphological changes that occur at developmental boundaries, such as metamorphosis or first exogenous feeding, may reduce the applicability of morphologically based descriptors of condition.

The lack of any strong relationships between specific aspects of the quality of newly settled *Upeneus tragula* and their length cautions against the direct transfer of size-based theories of the determinants of survival to

the variability found within a particular developmental stage. Studies that have examined condition through ontogeny have found that body size is a useful predictor of quality since it is closely related to developmental status, especially in the larval stages when growth and differentiation are rapid (e.g. Miller *et al.* 1988). However, the present study has shown that at a particular developmental stage, size looses its predictive ability. The high variability present in all the measures of condition, and the documented link between many of these measures and survival (e.g. Rosenberg & Haugen 1982, Bailey 1984, Folkvord & Hunter 1986, Litvak & Leggett 1992), suggests that this variability may be important in influencing which fish reach the reproductive population.

Chapter 6

Effects of feeding history on the growth characteristics of the reef fish, *Upeneus tragula*, at settlement

6.1 Synopsis

A feeding experiment was conducted on the pelagic stages of the tropical goatfish, *Upeneus tragula*, to examine how food quantity affects growth characteristics at settlement. Pelagic goatfish were collected from aggregation rafts 5 km west of Lizard Island on the northern GBR during December 1990. Three tanks in each of four feeding regimes were stocked with 25 pelagic goatfish between 20 and 23 mm standard length (SL). The four feeding regimes were: fed *ad libitum* (FED); fed once per day (F1PD); starved every second day (ST1D); starved for 3 d and re-fed *ad libitum* (ST3D). Fishes were fed 36 to 48 h old *Artemia* sp. nauplii (Ocean Star strain). Fish were removed from tanks when they underwent metamorphosis, changed pigmentation and settled to the bottom of the tanks. Morphology, muscle development, time taken to settle and biochemical condition were examined. Growth attributes of the experimental fish were compared to fish which settled within 24 h of capture (FIELD). All attributes examined were significantly influenced by the feeding treatments. Fish within the FED and ST3D treatments were significantly larger and heavier than fish in the less well-fed treatments (F1PD, ST1D). Similarly, concentrations of total lipid, carbohydrate and protein in the settled fish were significantly higher in the FED and ST3D treatments compared with the ST1D and F1PD treatments. For all these morphological and biochemical attributes the ST1D and F1PD fish did not differ significantly from the field fishes, but did differ from the FED and ST3D fishes. Water content was significantly higher in the F1PD, ST1D and FIELD fishes compared with the FED and ST3D fish. Furthermore, the average time taken to settle followed the pattern: FED (14 d) < ST3D (16 d) < ST1D (22 d) < F1PD (24 d). Muscle development mirrored the patterns in fish length among treatments, being more developed in the FED treatment and least well developed in the ST1D treatment. Feeding rate differed significantly among treatments and changed through the experiment for all but the FED treatment. Feeding rate decreased slightly for the F1PD and ST1D treatments over the experimental period, but increased rapidly for the ST3D treatment. The experiment suggests that *Upeneus tragula* are physiologically well suited to exploiting a patchy food source, and that food availability within the pelagic stages can have a major influence on the growth characteristics of this reef fish at settlement. The ramifications of these findings are discussed in relation to survival and success once fish have recruited to the reef population.

6.2 INTRODUCTION

The life cycle of a typical marine fish is characterised by several discrete life history periods. Both broadcast spawned and demersally laid embryos develop into free swimming pelagic larvae that subsequently metamorphose into juveniles, similar in morphology to the adults. In reef fishes, this metamorphosis is usually closely coupled with a major change in habitat, as the fish swim from the waters above the reef to join the demersal reef fish population. This settlement process is rapid for many reef fish, often occurring overnight (e.g. Sweatman 1985b, Youson 1988). For many it constitutes the greatest change in the suite of environmental (e.g. light attenuation, temperature fluctuations, salinity) and biological (e.g. acquisition of food, competition, predation) pressures that the reef fish will actively experience during its lifetime. Success in undergoing these major changes and surviving the new competitive and predatory forces on the reef is likely to be linked to the health of the fishes at settlement, and their size and developmental state.

Growth characteristics at settlement are reflections of growth and developmental rates up to that point. Few studies exist of the factors that govern the timing and physical condition of fish at metamorphosis (e.g. Laurence 1975, Policansky 1982, Seikai *et al.* 1986), and several of these have serious design flaws that hamper interpretation. No such studies are presently available for coral reef fish. The view that interannual variation in mortality rates during the early life stages is the principal regulator of recruitment variability (Hjort 1914, McGurk 1986, van der Veer 1986) has led most studies to concentrate on the embryo and early larval stages of temperate marine fish. These studies have identified the availability of planktonic food items as the major determinant of growth and mortality (Kiorboe *et al.* 1988, Pepin 1990). The potential of fishes to grow (as measured by the rate and efficiency of food consumption) has been found to depend on whether they have been deprived of food at any stage during their life history (Talbot 1985).

Hydrological features (e.g. slicks, internal waves, frontal systems, see Kingsford 1990) that aggregate food items (Kingsford & Choat 1986, Oliver & Willis 1987, Kingsford *et al.* 1991) are variable in extent and temporal duration. Ichthyoplankters can therefore be expected to experience periods of excess and paucity of food supplies, and variation in quantity and quality of food items available. Under this range of feeding regimes, from *ad libitum* to starvation, larval fishes may manifest different rates of somatic growth and sensory development. An understanding of how feeding history within the larval stages influences the body condition, size and age at settlement may enable us to

interpret the variability seen in the growth characteristics at settlement with respect to the processes that may have contributed to it.

The previous chapters have demonstrated the high variability present in the size, age and body composition of newly settled individuals of one species of reef fish, *Upeneus tragula*. Interestingly, much of this variability was explained by differences among samples collected at different times or locations, thereby suggesting an environmental influence. The present chapter is an experimental analysis of how the feeding history within the plankton affects the growth rate and somatic development of the goatfish, *Upeneus tragula*, through to settlement. In doing so, this experiment addresses the potential for the feeding history within the plankton to drive some of this variability.

6.3 MATERIALS AND METHODS

6.3.1 Sampling and experimental design

The study was conducted at the Lizard Island Research Station, on the northern GBR. Experimental fish were collected approximately 5 km west of Lizard Island, in 22 m of water. Fish were attracted to a series of 1 x 1 m plastic aggregation rafts moored for 2 h, and were sampled with a 14 x 2 m plankton mesh purse seine. Once fish were aggregated on the windward side of the dinghy in the pursed net, *U. tragula* of a similar size were scooped into 70-litre lidded black-plastic containers. These were then connected to a flow-through seawater supply and aeration system for transport back to the laboratory; this system was maintained back at the laboratory. At night, when the fish were torpid, fish which were visually judged to be of a similar size (approximately 20 to 23 mm SL) were placed into containers of aerated tetracycline-hydrochloride (250 mg/l) to reduce infection through scale loss. After 12 hours fish were randomly assigned to one of four feeding treatments. Presettlement mullids were found to be very susceptible to stress when first captured and, unless great care was taken during capture and transport, high mortalities resulted (often 95%). Thus, fish were not weighted or measured prior to the experiment. Tanks were screened from one another by black plastic, and were subjected to a natural light regime.

Three trials of four feeding treatments were conducted. All experimental tanks could not be set up with fish caught at the same time due to the unavailability of fish in the correct size range. Consequently, one trial was initially set-up, and the remaining two were initiated 16 d later. The feeding regimes used are thought to cover a realistic spectrum of food availability. A preliminary starvation trial resulted in mass mortality of fish after 6 d. The four feeding treatments were: fed continuously (FED); fed once per day (F1PD);

starved every second day (ST1D); and starved 3 d then re-fed (ST3D). Those fish which were to be fed more than once per day always had food in their tank during daylight hours. Fish were fed 36 to 48 h old *Artemia* sp. (Ocean Star strain) nauplii. For each of the two batches of fish used to set up the experimental trials, fish which had undergone metamorphosis and settled the night after capture were used as a field sample (FIELD). These were preserved for histological and biochemical examination.

Twenty-five fish were placed in each of three 60 litre tanks per treatment. At 0630 hrs each day, the tanks were examined for newly settled individuals; these were removed, rapidly killed by cold shock and processed as detailed below.

6.3.2 Variables measured

Morphometrics

Prior to preservation, a variety of measurements were made to assess the physical development of each fish: wet weight; total and standard length; body depth at the base of the pectoral fin and anal fin.

Age at Settlement

Since fish were randomly assigned to the feeding treatments, the tanks were assumed to contain fishes with a similar age distribution. The average time for the fish to settle in each tank was recorded and compared among treatments as this was indicative of the effect of the treatments on the age at settlement.

Muscle Development

Five fish from each of two tanks per treatment were preserved in formalin acetic acid calcium chloride (FAACC, Chapter 3 for recipe) for histological examination of striated muscle development. A cross section of muscle was removed at the start of the anterior dorsal fin, sectioned at 6 µm, and then stained with trichrome. Maximum muscle fibre diameter was then determined for fifty fibres within the second dorsal-most myotome (musculus carinatus dorsalis). The size distribution of muscle fibres from the treatment fish were compared to that from fish that settled in the collection bins the night after capture (FIELD).

Body Constituents

Following the removal of otoliths, the fish were stored in liquid nitrogen for later biochemical analysis of their body constituents; they were then freeze dried to a stable weight, yielding the water content and dry weight for each fish. The latter

Footnote 1: It should be noted that the variability in the genetic stock and age of the experimental fish suggest caution when comparing the initial field samples of settled fish with the treatment fish. The field samples, which metamorphosed 9 to 12 d earlier than the experimental fish, will have endured different pelagic life histories (e.g. different spawning and hatching temperatures and food levels at first feeding) and this alone may account for the differences observed.

measurement was used to calculate the volume of 100 m*M* sodium chloride solution required to maintain the sample loading (Hopkins *et al.* 1984). Each fish was homogenised in the NaCl solution and three aliquots of approximately 1.5 ml of the homogenate from each fish was taken and used for biochemical analysis. Total protein was estimated colorimetrically (Bradford 1976).

Carbohydrate content was determined by a modification of the method used by Mann & Gallagher (1985). A 1.0 ml aliquot of homogenate was placed in a 15 ml glass centrifuge tube and 1.0 ml of cold 0.6 *M* perchloric acid (PCA) was added. PCA was used instead of tri-chloracetic acid to prevent possible absorptancy interference (Munro & Fleck 1966). After mixing for 5 min and allowing to stand for 12 hours at 4 °C, the tubes were centrifuged at 1000 x *g* for 10 min and the supernatant collected. Cold 0.4 *M* PCA (1 ml) was added to wash the precipitate and the mixture was centrifuged at 1000 x *g* for 10 min The supernatant was collected and added to the supernatant collected previously. A 0.5 ml aliquot of the supernatant mixture was placed in a 20 ml glass vial. To this was added 1.0 ml of distilled water and 1.0 ml of 5 % w/v phenol solution. The vial was then mixed. Concentrated sulphuric acid (5 ml) was rapidly added to the mixture and mixed. The solution was allowed to cool for 30 min before being read on a spectrophotometer at 490nm.

A chloroform / methanol extraction (Mann & Gallagher 1985) was used to determine the lipid content of the treatment and field fish. Duplicates were run for each fish for each determination and appropriate standards and blanks were analysed in the spectrophotometer simultaneously.

Feeding rates

The feeding rate of pelagic *Upeneus tragula* was recorded for two tanks in each of the four treatments. The number of successful strikes at *Artemia* sp. over a 15 second interval was recorded for 10 fish per tank. Sampling was started 2 min after the commencement of the morning feed and strike rates were sampled three times per treatment throughout the course of the experiment. The timing of the three samples differed among treatments, and was in relation to the average time taken for fish to settle in each treatment: Sample 1, 2 d after the feeding of the ST3D treatment had commenced; Sample 2, when one-quarter of all the fish within the two tanks (for each treatment) had settled; Sample 3, when only 10 fish remained in either of the two tanks.

6.3.3 Analysis[1]

The differences among treatments and tanks nested within treatments were analysed with analysis of variance (ANOVA). Residual plots were examined and

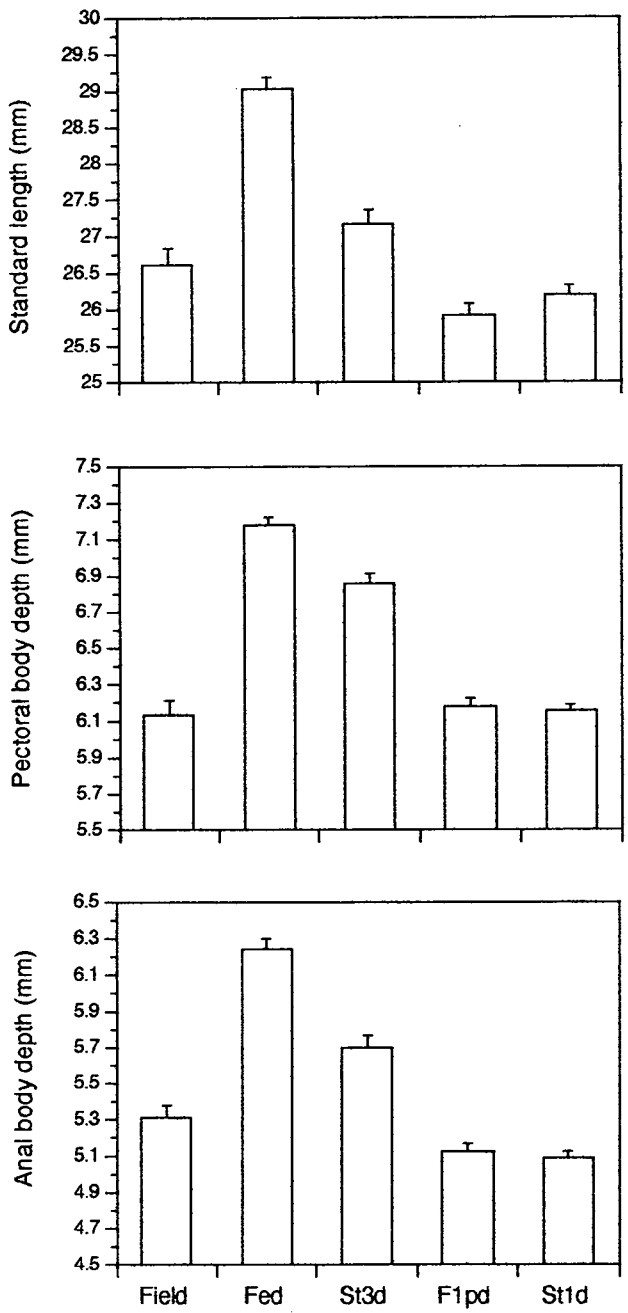

Figure 6.1. Comparison of mean standard length, depth at the pectoral fin and depth at the anal fin (mm) of newly settled *Upeneus tragula* among four feeding treatments and a field sample. Here and in later figures, error bars are standard errors.

data were found to conform to the analysis of variance assumptions of homogeneity of variance and normality. Where the number of fish within the tanks differed, type III sums of squares was used in the calculation of the mean square estimates (SAS/STAT 1987). The nature of the significant differences found by ANOVA were examined by Tukey's Studentized range test (HSD). The relationship between the field sample and treatment fish was examined at the tank-within-treatment level using ANOVA and Tukey's tests. For brevity in the presentation of the results these tests have not been included in the tables, however, results are interpreted with respect to these tests and significance levels are supplied in the text where appropriate. Any reference to an effect being "significant" refers to statistical significance at the alpha < 0.05 level.

The size distributions of muscle fibre maximum diameters was examined using maximum-likelihood estimates in an ANOVA framework (CATMOD, SAS/STAT 1987). For analysis, fibres were grouped into: $\leq$ 10; 10-15; 15-20; 20-25; 25-30; 30-35; 35-40; $>$ 40 μm.

6.4 RESULTS

6.4.1 Morphometrics

The three linear body measurements, standard length, pectoral and anal body depth of *Upeneus tragula* showed the same trends among treatments (Fig. 6.1). Those fish in the fed *ad libitum* treatment (FED) were consistently larger in all three body dimensions than either the 'fed once per day' (F1PD) or 'starved every second day' (ST1D) treatments. Fish within the 'starved for three days and re-fed' treatment (ST3D) were significantly smaller than the FED treatment, but larger than the F1PD or ST1D treatments. The FIELD fish were significantly smaller than fish from the FED and ST3D treatments, but did not significantly differ from either the F1PD or ST1D fish. There were no significant differences in the attributes among tanks within treatments (Table 6.1).

These trends were emphasised when fish weight was examined among treatments (Table 6.1, Fig. 6.2). *A posteriori* tests (Tukey's HSD) revealed that the FIELD fish did not differ significantly from the F1PD or ST1D treatments, but did differ from the FED and ST3D treatments. However, there were differences in fish weight among the three tanks within the treatments. Figure 6.2 shows that, although there were significant differences among tanks within treatments, the same general trends held for particular tanks across treatments. This difference among tanks stems from tank one, which was set up from fishes caught two weeks earlier than those that stocked the other two tanks.

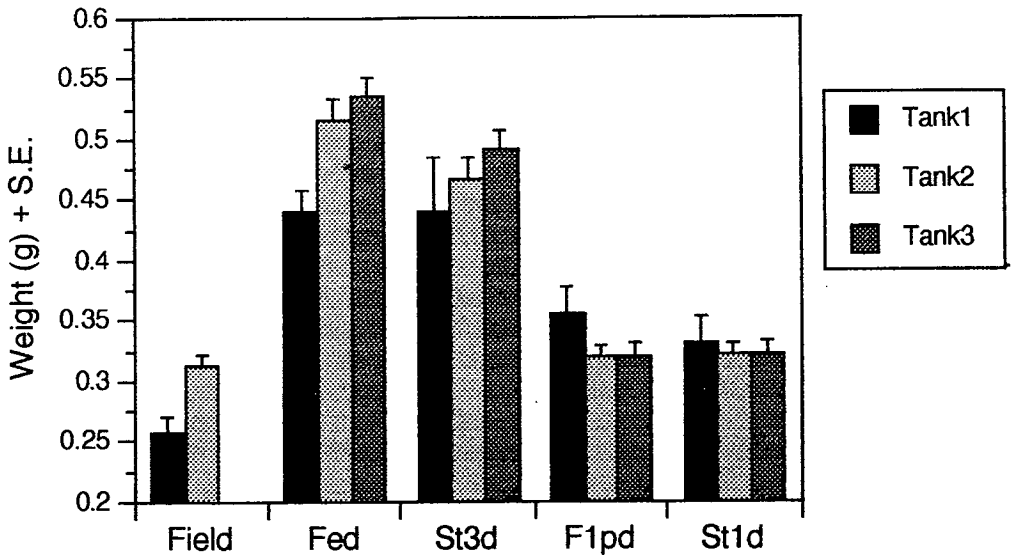

Figure 6.2. Comparison of the mean weight (g) of *Upeneus tragula* among tanks in four feeding treatments and two field samples. Tank 1 "field", represents fish that settled overnight upon capture from the sample of fish that stocked experimental Tank 1; Tank 2 "field", represents settled fish from the sample that stocked Tanks 2 and 3.

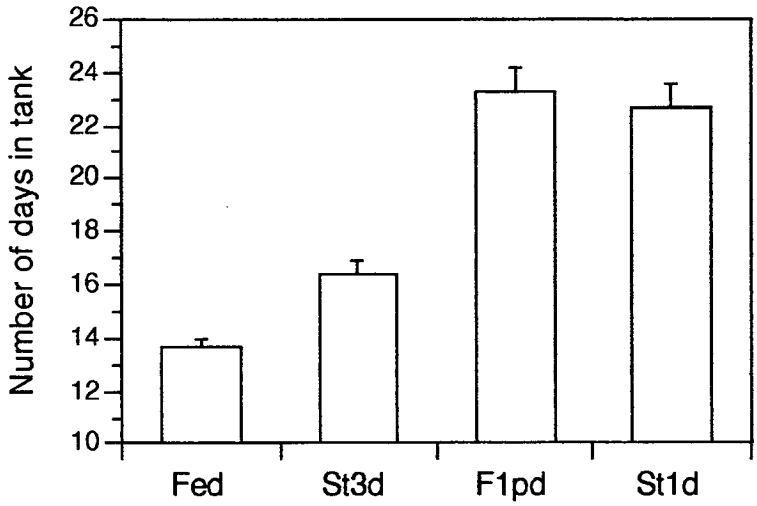

Figure 6.3. Comparison among four feeding treatments of the mean number of days that *Upeneus tragula* were in tanks before they settled.

Table 6.1. Summary of analyses of variance comparing various morphological, biochemical and behavioural attributes of the goatfish, *Upeneus tragula*, held until settlement within replicate tanks, under four feeding treatments. Data are not transformed.

Attribute	Source	DF	MS	*p*
Standard length	Treatment	3	112.33	<0.0001
(mm)	Tank(Treat.)	8	2.42	0.1608
	Residual	220	1.62	
Weight	Treatment	3	0.3955	<0.0001
(g)	Tank(Treat.)	8	0.0143	0.0045
	Residual	220	0.0049	
Time in tanks	Treatment	3	1236.91	<0.0001
(d)	Tank(Treat.)	8	37.26	0.2327
	Residual	220	28.15	
Lipid content	Treatment	3	17744.12	<0.0001
(mg/g wet wt)	Tank(Treat.)	8	545.24	0.1140
	Residual	101	325.80	
Carbohydrate content	Treatment	3	43.19	0.0042
(mg/g wet wt)	Tank(Treat.)	8	4.31	0.1005
	Residual	101	2.93	
Protein content	Treatment	3	938.67	0.0016
(mg/g wet wt)	Tank(Treat.)	8	69.35	0.1509
	Residual	101	44.86	
Water content	Treatment	3	8664.50	<0.0001
(mg/g wet wt)	Tank(Treat.)	8	207.20	0.0545
	Residual	101	103.86	
Bite rate	Time	2	85.10	0.0460[a]
(strikes/15 s)	Treatment	3	2777.49	<0.0001[a]
	Time X Treat.	6	674.02	<0.0001[a]
	Tank(Treat.)	4	28.41	0.9779
	Time X Tank(Treat.)	8	28.41	0.9635
	Residual	216	28.41	

[a] Calculated from pooled, nested, tank terms.

6.4.2 Age at settlement

The number of days taken for fishes to settle within the tanks differed significantly among treatments, but not among tanks within treatments (Table 6.1, Fig. 6.3). *Upeneus tragula* from fed tanks settled significantly faster than fish from any of the other treatments; they underwent metamorphosis and settled in an average of 14 d, compared to fishes within the F1PD treatment which took

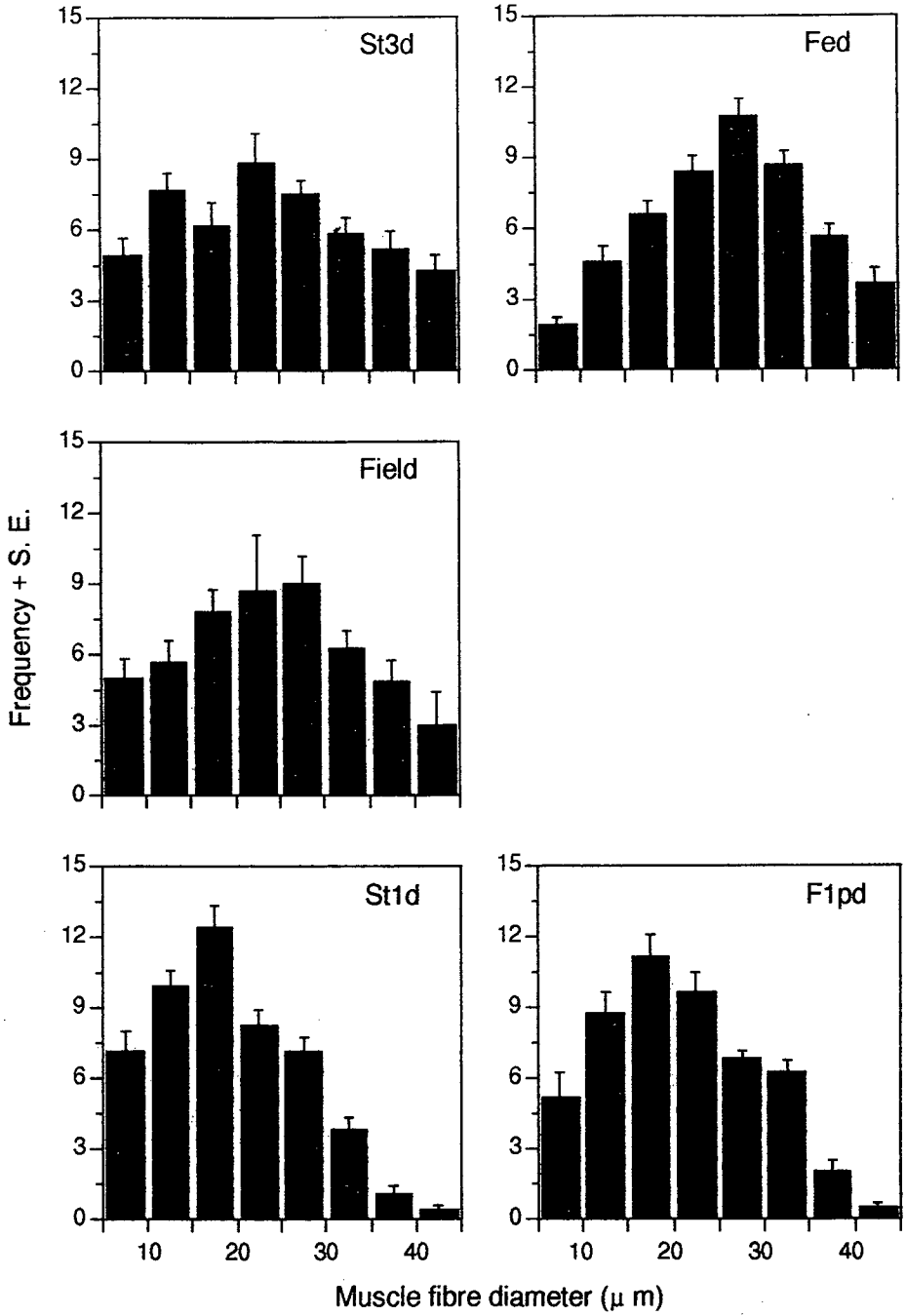

Figure 6.4. Maximum muscle-fibre diameter size frequencies (5 μm size classes) for newly settled *Upeneus tragula* from a field sample and samples from four feeding treatments (n = 10).

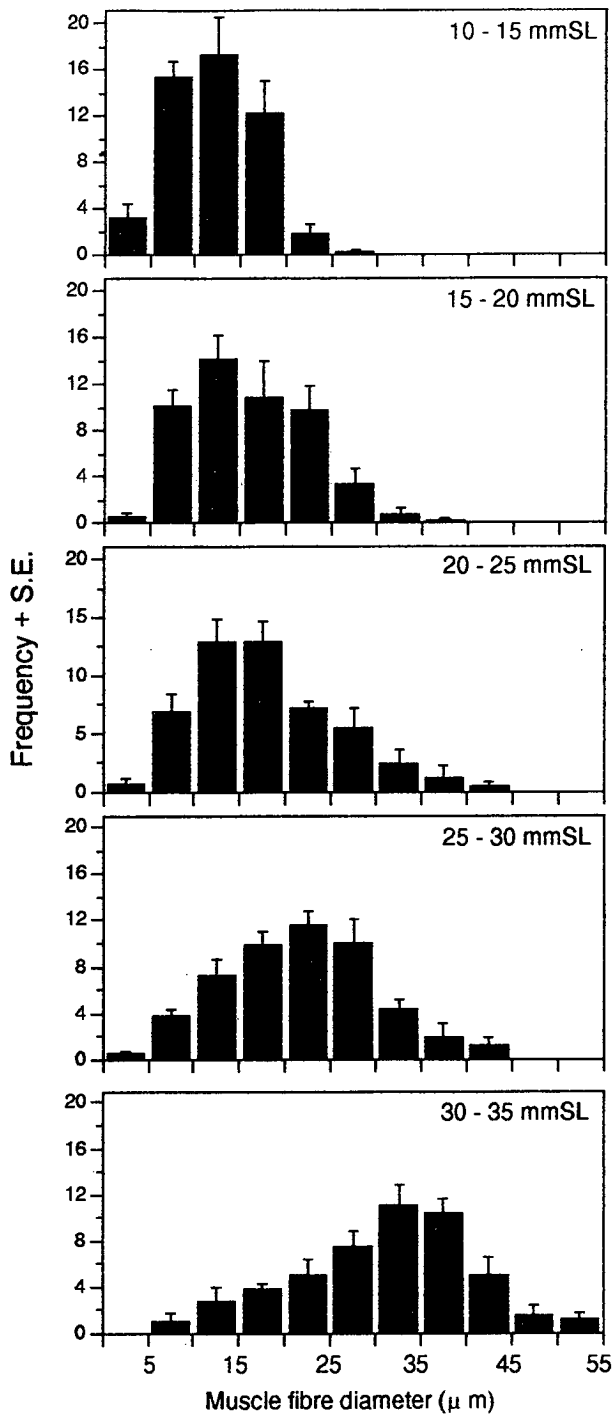

Figure 6.5. Ontogenetic series of maximum muscle-fibre diameter distributions for 5 μm size classes, from field caught *Upeneus tragula* (n = 5).

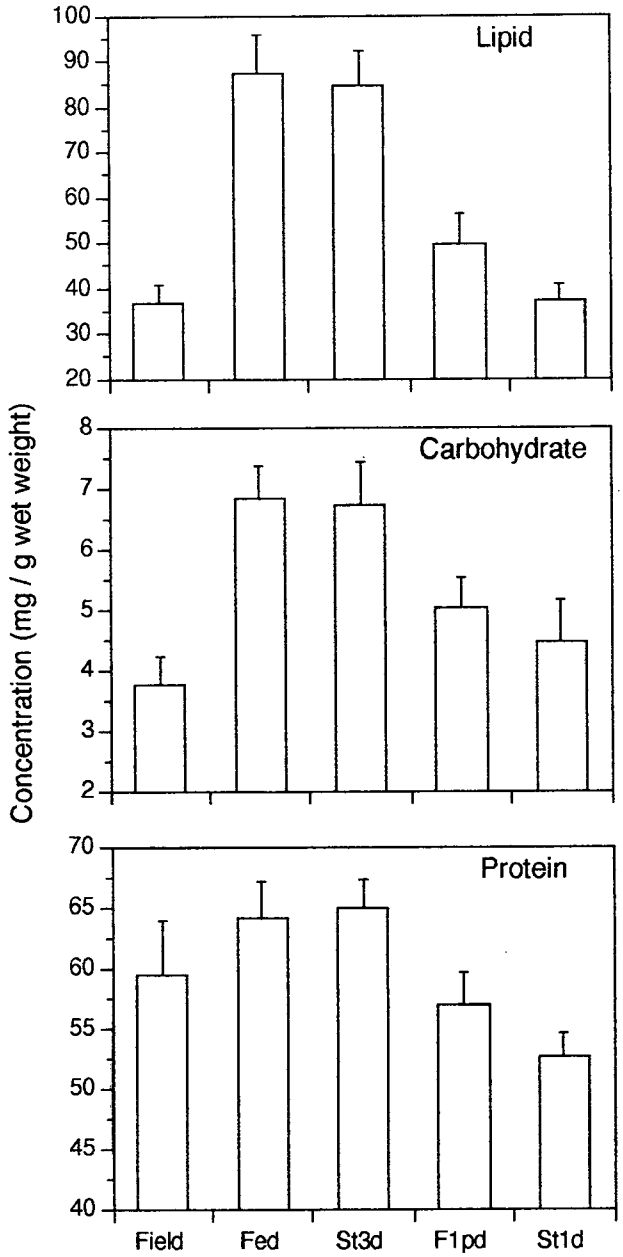

Figure. 6.6. Comparison of mean total lipid, carbohydrate and protein content (mg/g wet weight) among samples of newly settled *Upeneus tragula* from the field and four feeding treatments.

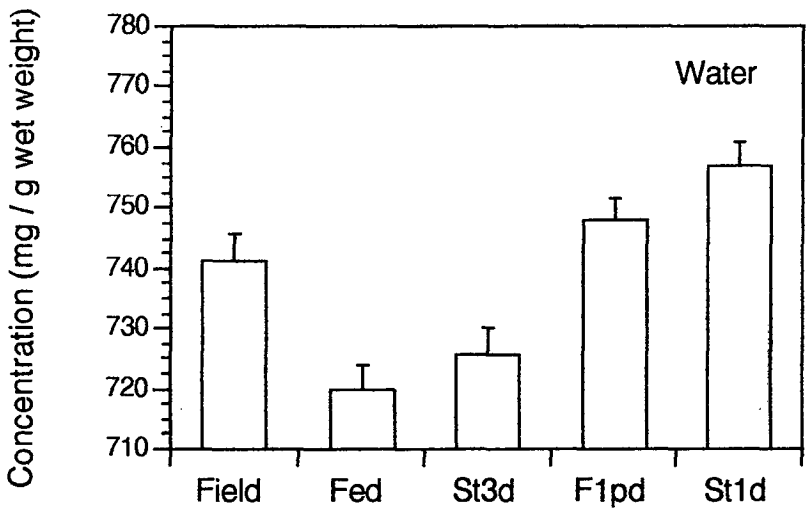

Figure 6.7. Comparison of mean water content among samples of newly settled *Upeneus tragula* from the field and four feeding treatments.

approximately 24 d. Fish within the ST3D treatment took only slightly (albeit significantly) more time to settle than those in the fed treatment (16 d).

6.4.3 Muscle Development

The size distributions of the muscle fibres differed significantly among treatments (Table 6.2). The ST1D and F1PD treatments had a predominance of small diameter muscle fibres, with a modal sizeclass of 15 to 20 μm (Fig. 6.4). In contrast, fish within the FIELD and ST3D tanks had a muscle size distribution which was almost symmetrical around the 20 to 30 μm size classes, whereas the fed treatment displayed a skewed fibre-diameter distribution, with a predominance of larger fibres and a modal size class of 25 to 30 μm. Muscle size distributions did not differ for fish within tanks, or tanks within treatments (Table 6.2). The state of muscle development was strongly related to the size of the fish. This is demonstrated when the muscle size-frequencies from the treatments are compared to an ontogenetic series (c.f. Fig. 6.4 and Fig. 6.5). Muscle distributions from the less well-fed treatments (F1PD, ST1D) were most similar to field sampled fish between 20 and 25 mm SL; the FIELD and ST3D distributions mirrored the 25 to 30 mm SL distributions; while the FED fish had muscle distributions between those of the 25 to 30 mm and 30 to 35 mm SL distributions.

Table 6.2. *Upeneus tragula*. Summary of maximum likelihood analysis of variance comparing size distributions of muscle fibre diameters among five fish within two tanks within four feeding treatments.

Source	DF	χ^2	*p*
Treatment	18	173.69	<0.0001[a]
Tank(Treat.)	24	22.80	0.5314
Fish(Treat. X Tank)	192	208.00	0.2037

[a] Calculated from pooled lower order factors

6.4.4 Body constituents

Differences in the body chemistry of fish within the four treatments and field sample were emphasised in the concentration of lipid (Fig. 6.6). Here, the fish within the fed treatment contained over twice the average amount of lipid of fish that had been starved every second day. Furthermore, there was a 6.5-fold maximum difference in lipid levels between fish within these two treatments (146 mg/g FED vs 21.9 mg/g ST1D). As with the fish morphology and muscle size distribution, there was no significant difference between the FED and ST3D

treatments or between the F1PD and ST1D treatments, but the two pairs of treatments differed significantly from one another (Fig. 6.6). There was no significant difference among tanks within treatments (Table 6.1). *A posteriori* tests (at the tank by treatment level) revealed that the field fish did not differ significantly in lipid content from fish in the ST1D and F1PD treatments.

The carbohydrate levels exhibited exactly the same patterns of significance, although less accentuated. Carbohydrate levels in fish within the FED and ST3D treatments were on average a third higher than those within the F1PD and ST1D treatments. The FIELD fish had the lowest mean levels of carbohydrate but these did not significantly differ from the F1PD and ST1D treatments (see Fig. 6.6).

Protein levels exhibited a slightly different pattern from those of lipid and carbohydrates. Concentrations within the FED and ST3D treatments were highest and did not significantly differ from one another. Both were significantly higher than the F1PD and ST1D treatments, which also differed from one another (Fig. 6.6).

Water content (Fig. 6.7) exhibited an inverse pattern to that of the lipid and carbohydrate levels. The fish within the FED and ST3D treatments exhibited the lowest water contents and were not significantly different from each other. Fish within the F1PD and ST1D treatments displayed the highest water contents and were not significantly different from each other or the FIELD fish.

6.4.5 Feeding Behaviour

Feeding rate was sampled with respect to the time taken for the treatment effect to manifest itself. This was thought to be more ecologically meaningful than sampling at fixed time intervals, since average time taken to settle within each treatment was related to developmental schedules. Within 5 d of the start of the experiment, bite rates 2 min after initial feeding differed significantly differ among the feeding treatments (Table 6.1, Fig. 6.8). The FED treatment displayed significantly higher rates than the other treatments, while the F1PD was significantly higher than the ST3D treatment, but did not significantly differ from fish in the ST1D treatment. The bite rate of fish within the fed *ad libitum* treatment did not change through the sampling period. On the other hand, fish within the F1PD and ST1D treatments reduced their bite rate, although this change was not significant for fish in the F1PD treatment. Fishes within the ST3D treatment showed the most dramatic change in bite rate over the

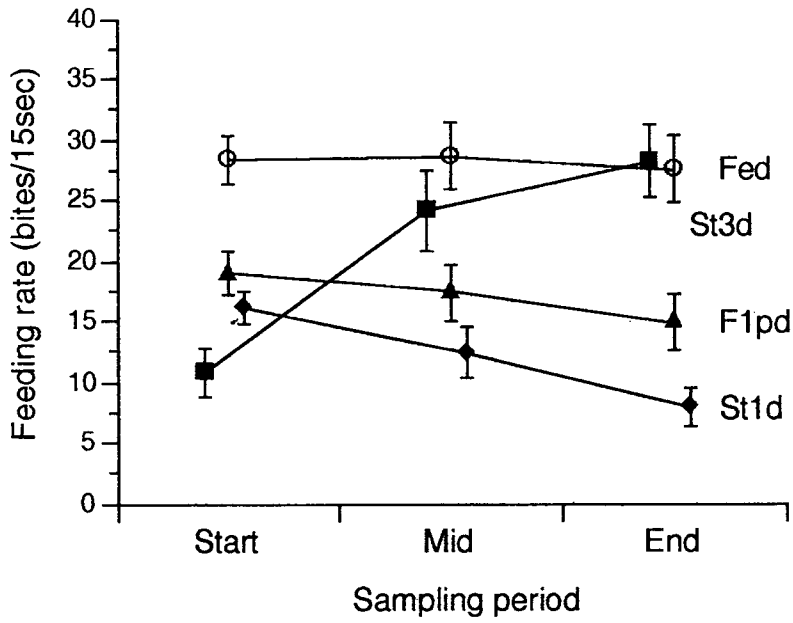

Figure 6.8. Mean feeding rates with 95% confidence limits for *Upeneus tragula* within four feeding treatments over the experimental period. "Start" represents two days after feeding of the ST3D treatment had commenced; "Mid", when one-quarter of all the fish in two tanks (for each treatment) had settled; "End", when only ten fish remained in either of the two tanks.

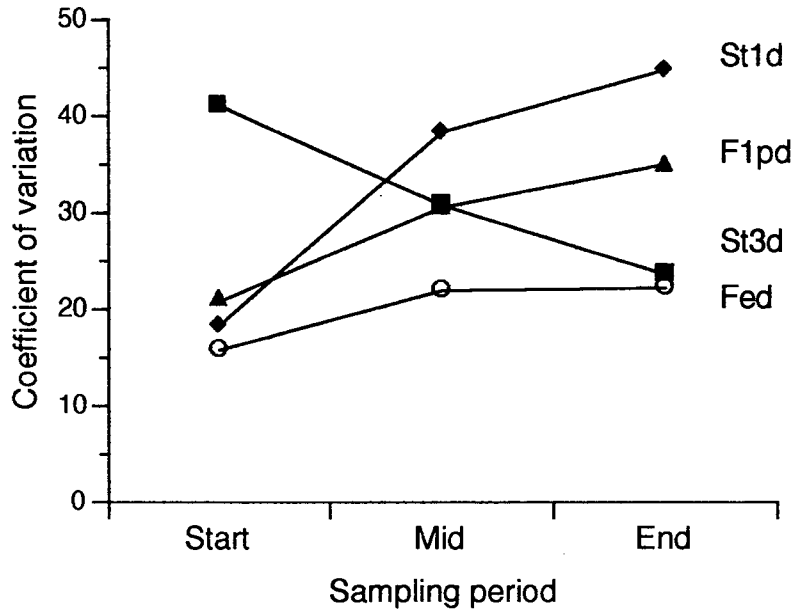

Figure 6.9. Change in the coefficient of variation in the feeding rates of *Upeneus tragula* within the four feeding treatments over the experimental period.

experiment, with a 3-fold increase in bite rate after the initial sampling period, 2 d after they had started being re-fed (Fig. 6.8).

Interestingly, the amount of variation around the mean bite rate increased over the experiment for all but the ST3D treatment (Fig. 6.9). Those treatments which received the least food had the highest variability in bite rate (ST1D > F1PD > FED, Fig. 6.9). In contrast, the ST3D treatment started the experiment with a coefficient of variation in bite rate twice that of the other treatments, and ended it with the lowest equal level of variability.

There was not only a difference in the feeding rate but also in the quantity of food eaten. Tank observations revealed that fish within the F1PD treatments visibly gorged themselves when fed, resulting in a highly distended gut for some hours after feeding, compared to the FED, ST3D or ST1D fish.

6.5 DISCUSSION

Few studies have examined in detail the growth characteristics of newly settling fishes. Research has concentrated on groups, such as the Pleuronectiformes and those that have leptocephalus larvae (e.g. Elopiformes, Anguilliformes), which undergo major somatic and physiological reorganisation during the metamorphosis associated with settlement (e.g. Pfeiler 1986, Youson 1988). Most have found that there is considerable variability in many of the growth characteristics measured (e.g. Policansky 1982, Ahlstrom *et al.* 1984, Pfeiler 1986, Chambers & Leggett 1987). This is exemplified in the Japanese flatfish *Limanda yokohamae*, in which the range in size at settlement can span 60 % of the maximum size at which the fish settles (Fukuhara 1986). Similarly, high levels of variability were noted in the age at which this fish underwent its last metamorphotic stage to settlement (Fukuhara 1986,1988). Most reef-associated fishes, such as the goatfish *Upeneus tragula* studies here, undergo more subtle changes at settlement. However, *U. tragula* has been found to have levels of variability in age and size at settlement similar to that of flatfish (Chapter 4).

An examination of the factors that influence these levels of variability is important to the understanding of the dynamics of reef fish populations. Variable larval growth and developmental rates have the potential to drive, not only the age and size at settlement, but also the number of settling fish and their subsequent survival. Miller *et al.* (1988), Houde (1989a, b) and Davis *et al.* (1991) all suggested that year to year variability in number of fish recruiting to a population could be largely governed by small changes in the daily growth and mortality rates of larvae within the plankton, possibly driven by factors such as

food abundance or temperature. In the present experiment, feeding history in the early pelagic stage of *U. tragula* was found to influence the growth characteristics at settlement. Fish that fed well during the pelagic stage were larger, heavier, could potentially swim faster and had more energy reserves at settlement than less well fed fish.

Specifically, the striated muscle of fish which received small quantities of food (F1PD, ST1D) was less well developed than those fish receiving unlimited food. Development appears to be inextricably linked to fish size, with larger, although younger fish having more developed muscles than smaller older fish (Weatherley & Gill 1985, Weatherley *et al.* 1988). In mammals and birds, the number of muscle fibres does not increase after embryonic differentiation, and a disruption during the tissue differentiation can determine the maximum size the tissue will obtain (Goldspink 1980). In contrast, Weatherley & Gill (1987) found that muscle growth in fish was inherently conservative with disruptions to development (through starvation etc.) offsetting muscle growth without reducing its growth rate once normal conditions had been restored. They found that it was not possible to distinguish between the modal diameter (or range) of muscle fibres of rainbow trout of the same size but that had grown at very different rates (Weatherley & Gill 1981, 1987). This was interpreted to mean that the swimming abilities would always be similar regardless of any difference in the time required to grow to that size. This suggests that for settling *Upeneus tragula*, the *growth potential* of a fish that had a low growth rate prior to settlement due to a poor food supply is not permanently affected. Thus, although a large fish begins its post-settlement existence with a growth advantage, a smaller fish if it is an efficient competitor or food is abundant, may reach a similar maximum size.

Nonetheless, this experiment has shown that *Upeneus tragula* that fed well in the pelagic life stages were larger at settlement than those that fed less well. This size advantage may be translated into a competitive advantage once they settle onto the reef. The outcome of many behavioural interactions, prey selection and entrance into the reproductive population are often size dependent (Peters 1983, Werner & Gilliam 1984, Jones 1987, Pepin *et al.* 1987, McCormick 1989, Pepin 1991). Ochi (1986) found a wide range of sizes of *Amphiprion clarkii* recruits (15 - 47 mm) at the end of the main settlement season. This difference was attributed to "differences in the duration of the first growing season spent after settlement and a social inhibition of growth of the subordinate late settlers by frequent attacks by the dominant early settlers". Large sized recruits were competitively dominant and this was later translated to a breeding advantage once reproductive sites had been vacated by dominant adults. However, Ochi assumed that the fish settled at a similar size and that the

size range he subsequently recorded was purely the result of post-recruitment processes. The alternative hypothesis, which was not examined, is that some of the recruits to the anemones started their benthic life with a size advantage due to feeding history within the plankton, and this was simply emphasised by competition after settlement.

Large size of a newly settling fish does not necessarily confer an immediate advantage. The outcome of interactions are more complex, being related to other factors such as the presence of conspecific adults (e.g. Sweatman 1985a). Jones (1987) conducted two experiments to examine the effects of recruitment and subsequent intraspecific competition on a population of the tropical damselfish *Pomacentrus amboinensis*. He found that adults primarily affected the growth of the initially largest, behaviourally dominant juveniles. Furthermore, there is some evidence for larval fish that larger individuals may be selectively preyed upon by predators because they are more conspicuous (e.g. Litvak & Leggett 1992). Whether size of a fish influences its probability of survival shortly after settlement is yet to be examined for reef fish.

In this experiment the well fed fish not only attained a size advantage at settlement but also derived key developmental benefits from their feeding regime. The larger modal muscle fibre diameter and higher protein levels suggests they may be faster and stronger swimmers (sustained swimming, even if not burst, see Chapter 5) than less well fed fish. Furthermore, by having larger storage reserves there is possibly greater flexibility in the timing of settlement, or habitat selectivity at or after settlement. Lastly, the higher bite rates suggest that these fish would be more efficient feeders once they had settled onto the reef. It is notable that, although fish collected at different times did differ in the magnitude of their response to the feeding treatments, the relative difference among treatments within particular batches of fish stayed the same. This suggests that the response patterns to food quantity shown in this experiment are likely to hold across cohorts.

It is interesting to note that significant and often dramatic effects were found in the growth characteristics of the experimental fish, even though the fish used were in a fairly advanced stage of their pelagic life history. It is likely that a more subtle range of feeding treatments may have equally dramatic effects on the fish just after exhaustion of the yolk sac, when lipid reserves are lower and the potential to survive environmental fluctuations considerably less. Moreover, the somatic effects of limited rations are likely to depend on whether the stress coincides with a critical period in the embryology, neurobiology or ethology of the fish. Blaxter (1970, 1988) and Browman (1989) hypothesised that if a larval fish does not obtain sufficient food for the duration of a sensitive period in neural

development, the appropriate neural network underlying foraging behaviours may not develop optimally even if food abundance subsequently increases.

With respect to the physiological flexibility of the young fish to respond to fluctuations in food availability, the response of fish within the "starved for 3 days and re-fed" treatment is of particular interest. The feeding rate of these fishes recovered rapidly, and within 12 d they were feeding at a similar rate to that of fish that had been fed continuously. Furthermore, the average number of days to settlement was only 3 d behind that of the FED individuals and settlement was at a size that did not differ from the continuously fed fish. This indicates that the growth potential of the fish had not been perturbed by the period of starvation. Furthermore, the biochemical assay suggest that these fish recovered well in body constituents, having high levels of storage lipids, proteins and carbohydrates. All evidence from this treatment points to this species being physiologically suited to coping efficiently with a patchy food resource. This suggests a resilience to an unpredictable food source, and that there will be a myriad of feeding histories in an environment with a patchy prey resource that will lead to the same biochemical and growth end point for a reef fish at settlement.

The lack of knowledge about the larval ecology of reef fish is emphasised by this study. If larvae move actively or passively in patches through the water column and periodically meet up with high density food patches, then a larval size-distribution which is skewed towards large individuals will eventuate if a patch of larvae meet up with these food patches at a greater than average frequency. This skew will occur whether or not intra-patch competition occurs at this larval stage; though the skew will be accentuated if competition is an important factor. Fish that have fed well in the plankton may be larger at a given age regardless of the action of intra-cohort competition. It is of importance to know whether they are more efficient competitors just because the fish are larger when they settle, or whether this is only a temporary advantage due to the lack of a socially aggressive behaviour which must be manifest in a competitively dominant individual.

In summary, this study suggests that food availability within the pelagic life stage can have dramatic effects on the growth characteristics of the fish that settle on the reef. By midway through the larval stage, this particular reef fish appeared relatively resilient to periods of starvation and showed a capacity for rapid recovery, which suggests they are well suited to exploiting a patchy food resource. Water temperature during larval development has also been found to be important in determining growth rates and mortality in the early larval stages of marine fish, and this may be important for settling reef fish. The relative

importance of food availability to water temperature is examine in the following chapter.

Chapter 7

Effects of temperature during the pelagic life stage on the growth characteristics of the reef fish, *Upeneus tragula*, at settlement

7.1 Synopsis

The effects of water temperature during the pelagic life-history phase on growth characteristics of the goatfish, *Upeneus tragula,* at settlement were examined. Water temperatures at three stations across the northern GBR were recorded during April 1989 - May 1990 and found to increase from 25 °C to 30 °C over the main recruitment season for reef fish (Oct. - March). An experiment examined whether water temperature during the larval phase influenced size and age at settlement, which previous chapters have shown to be highly variable, by the influence of water temperature on larval growth and developmental rates. Late-pelagic stage reef fish (20 - 23 mm SL) were chosen at random and placed in three tanks at each of two temperatures, 25 and 30 °C. Fish were fed *ad libitum* and removed from tanks upon settlement. The length, weight, muscle development and biochemical composition did not differ between temperature regimes. However, the time taken to settle did differ between treatments. Those fish in the 30 °C water settled on average 2.7 days faster than those in the 25 °C tanks. The findings are discussed in relation to the relative influence of water temperature and food availability on the size characteristics of reef fish at settlement. Temperature was found to be of secondary importance to food availability in governing the relationship between somatic growth and differentiation.

7.2 INTRODUCTION

In empirical analyses, water temperature is the environmental variable most frequently linked to the recruitment variability of temperate marine fish (Sissenwine 1984). The effects of water temperature on the embryo and yolk-sac stages, to which most of pre-recruitment mortality is attributed, has historically been the subject of considerable interest. Temperature before spawning has been shown to be inversely related to the size of eggs produced (Bagenal 1971, Ware 1975, Tanasichuk & Ware 1987), and time to starvation in the early larval stages is temperature dependent (Houde 1974). Furthermore, water temperature directly influences the conversion efficiency of yolk into energy for growth, assimilation rates and rates of mortality (e.g. Fukuhara 1990, Houde 1990). Through the direct influences of temperature on these life history processes, and its interaction with the phytoplankton production cycle, temperature has been posited as a major environmental forcing agent governing cohort success (Houde 1989 a, b).

The emphasis of these studies has usually been on cohort survivorship, and the importance of the average size and age of fish in the target population (e.g. Rijsdorp *et al.* 1992). Only recently has attention focused on the influence of temperature on the variability around these population parameters (e.g. Chambers & Leggett 1992). Few studies have addressed how temperature influences the rate processes that drive the variability in size, age or body condition of fish larvae. The link between growth and development can be a dynamic one (Chambers & Leggett 1987, Chapter 3, 4, 5) and is likely to be affected by temperature. An understanding of what contributes to the variability in body characteristics at a particular developmental stage is important since these characteristics may determine the fishes' subsequent mortality and growth schedules. For demersal fish in which a change in habitat is generally closely associated with metamorphosis, this variability in growth attributes may be of paramount importance.

For the goatfish *Upeneus tragula*, there is considerable variation in all the features commonly used as indices of quality and condition (Chapters 4, 5). This is not unusual for demersal reef fishes. For instance, Wellington & Victor (1992) found regional variation in the age and growth rates to settlement (and metamorphosis) of a species of wrasse (*Thalassoma lucasanum*) and 2 species of damselfish (*Stegastes flavilatus*

and *Microspathodon dorsalis*) in the tropical eastern Pacific. The growth rates through to metamorphosis were found to differ by 1.5 times among four sampling sites spread over 3500 km. A knowledge of the influence of temperature on growth rates through to metamorphosis may aid the interpretation of the high variation in body attributes seen in the field.

The present study examines experimentally the influence of water temperature on the growth characteristics at metamorphosis of the tropical goatfish, *Upeneus tragula*, over a temperature range normally encountered in nature. The two principal objectives were to: (1) examine whether temperature influenced the relationship between size and age at settlement, and thereby processes of growth and differentiation; (2) examine whether temperature influenced the body condition of fish at settlement. The importance of water temperature is weighed against the importance of food availability in governing these life history traits.

7.3 MATERIALS AND METHODS

7.3.1 Water temperature

Pelagic stage *Upeneus tragula* occur commonly in all areas of the GBR lagoon (Chapter 2). Although the main period for settlement is between October and March, recruitment to the reef does occur in low numbers throughout the year. Therefore, water temperature data were collected at three locations across the northern GBR: an inshore station at the Turtle Group; a mid-shelf station at Lizard Island; and a Outer Barrier station at the Cod Hole (Fig. 2.2). Data were collected by the staff of the Lizard Island Research Station, using calibrated dataloggers (HUGRUN Seamon UTR-B) at depths of 10 - 12 m.

7.3.2 Temperature experiment

The study was conducted at the Lizard Island Research Station from 8th Jan 1991 to 27th Jan 1991. Experimental fish were collected approximately 5 km west of Lizard Island, in 22 m of water (Fig. 2.2). Fish were attracted to a series of 1 x 1 m plastic aggregation rafts that were moored for 2 h and then sampled using a 14 x 2 m plankton-mesh purse seine (0.5 mm mesh). Once captured they were transported back to the laboratory as detailed in section 6.3.1. Once in the laboratory, pelagic-stage *Upeneus tragula* between 20 - 23 mm SL, were placed in aerated containers of tetracycline hydrochloride in seawater (250 mg/l). This treatment reduced the

susceptibility of the fish to infection as a result of scale loss. After 12 h, fish were randomly assigned to one of two temperature treatments: 25 °C and 30 °C. These temperatures represented the range of the mean daily water temperatures over the main recruitment season (see section 7.4.1). Three tanks were set up for each treatment. A refrigeration coil in a header tank reduced the water temperature of the flow-through seawater from an average of 28 °C to a constant 25 °C. Water heaters kept the other tanks at 30 °C. Fish were fed *ad libitum* 36 - 48 h old *Artemia* sp. nauplii (Ocean Star strain). Twenty fish that had undergone metamorphosis and settled to the bottom of the tank the night after capture were used as a field sample for comparison with the treatment fish. Morphological characteristics were measured for all twenty fish. Ten of these fish were prepared for biochemical and ten for histological comparisons.

Twenty fish were placed in each of three 20 litre tanks per treatment. At 06.30 hrs each day, prior to feeding, tanks were examined for newly settled individuals. These individuals were removed and components processed as detailed below. Processing methods are similar to those used in the feeding experiment (Section 6.3.2).

Briefly, the following data were recorded upon settlement:

(i) *Morphometrics*: wet weight, total and standard length, body depth at the base of the pectoral fin and anal fin;

(ii) *Age at settlement*: measured as the number of days taken by an individual fish to settle in a tank;

(iii) *Muscle development*: measured as the size distribution of 50 muscle fibres from histological sections of five fish from each of two tanks per temperature treatment. Size distribution of muscle fibres were compared to those of fish that settled in the collection bins the night after capture.

(iv) *Body constituents* measured as total lipids, carbohydrates, proteins and water content (mg / g wet weight) were obtained for 7 - 10 fish from each treatment tank and the field sample.

(v) *Feeding rates*

The feeding rate of these captive fishes was recorded for the three tanks in each of the two temperature treatments. The number of successful strikes at

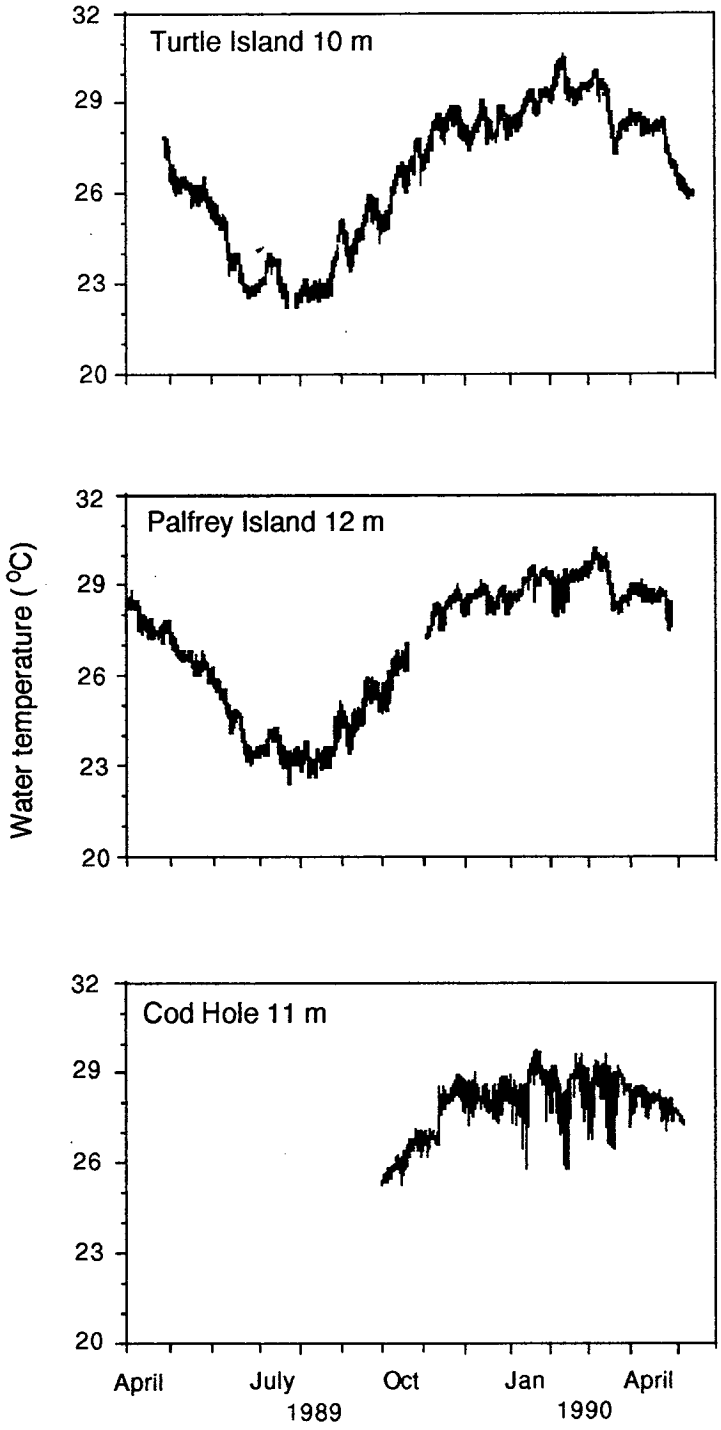

Figure 7.1 Water temperature at three stations across the northern Great Barrier Reef at between 10 - 12 m depth.

Artemia sp. over a 15 s interval was recorded for 10 fish per tank. Recording began two minutes after the start of the morning feeding of the fish. Strike rates were sampled three times per treatment through the course of the experiment. The timing of the three data recordings differed between treatments, and related to the average time taken for fish to settle in each treatment: Sample 1, one day after the start of the experiment; Sample 2, when approximately one-quarter of all the fish within the 3 tanks (for each temperature) had settled; Sample 3, when approximately 10 fish remained in each of the treatment tanks.

7.3.3 Analysis

Statistical analysis follows that of the feeding experiment (section 6.3.3). Only one sample of the field population was required to quantify the variation in the newly settled *Upeneus tragula* from the sample of fish used to stock the treatment tanks. However, this complicated analysis slightly, since three tanks were nested within each of the experimental temperature treatments. Thus two series of analyses were undertaken. The main test for differences between temperature treatments was analysed with a two factor (temperature, tanks) nested analysis of variance (ANOVA). These are the analyses presented in results table. The relationship between the field sample and treatment fish was examined at the tank-within-treatment level (the Field sample being analogous to a tank population) using ANOVA and Tukey's Studentised range tests (HSD). For brevity in the presentation of the results these tests have not been included in the tables, however, results are interpreted with respect to these tests and significance levels are supplied in the text where appropriate. Graphs (with standard error bars) are provided for each comparison. Any reference to an effect being "significant" refers to statistical significance at the alpha < 0.05 level.

7.4 RESULTS

7.4.1 Water temperature

The 3 stations across the northern GBR lagoon showed similar trends in temperatures over a year (Fig. 7.1). The minimum temperature at 10 - 12 m depth for 1989 to 1990 was 22.0 °C at the inshore Turtle Island station, and 22.6 °C at the midshelf Palfrey Island station, both of which occurred in late July 1989. The maximum temperatures were 30.8 °C during early February at the Turtle Station, 30.0 °C in early March at the Palfrey Station, and 29.7

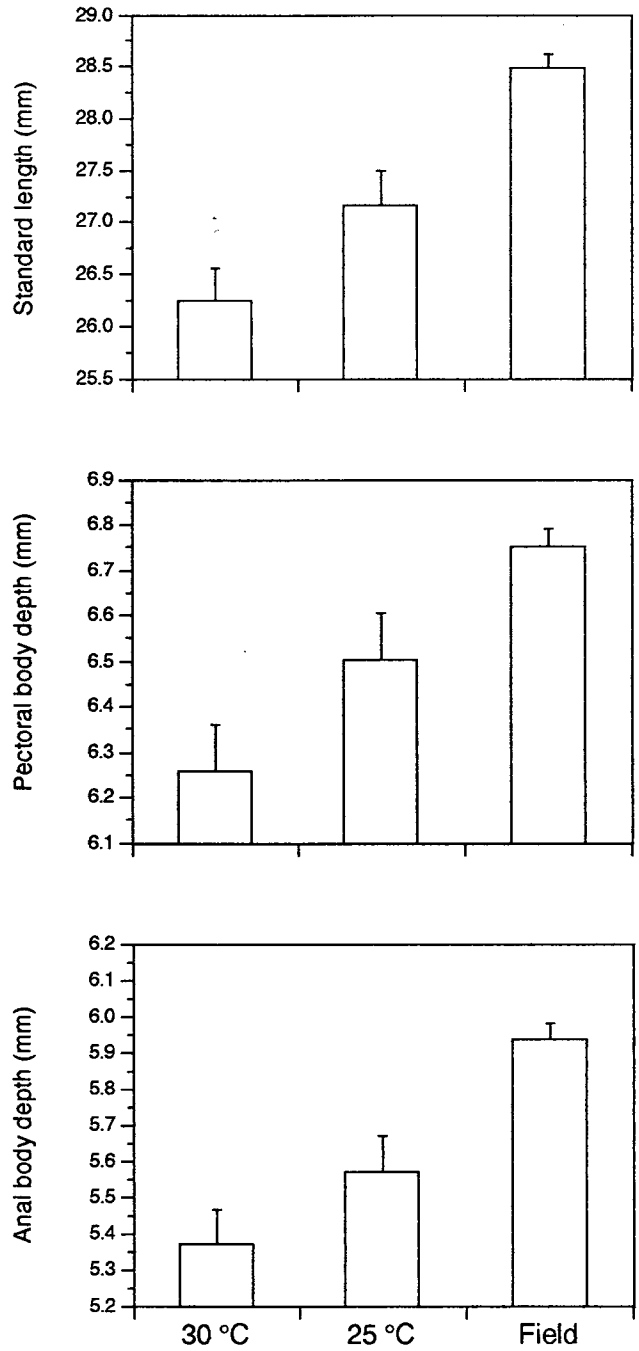

Figure 7.2. Comparison of the standard lengths (mm), body depth at the pectoral fin and anal fin (mm) of newly settled *Upeneus tragula* among two temperature treatments and a sample of field fish. Error bars are standard errors.

°C at the Cod Hole in January and February. At the Cod Hole station large daily fluctuations in the water temperature were associated with the spring tides in January through to March 1990, e.g. during mid-February when a range of 3.1 °C was recorded over a tidal cycle (25.8 - 28.9 °C). This large daily temperature range was a localised phenomenon, probably involving the pumping of cooler water from below the thermocline into the surface layers by tidally-driven Bernoulli suction (Wolanski *et al.* 1988). Daily ranges for the other stations between January and February 1990 were on average 0.6 °C at Palfrey Is. and 0.5 °C at the Turtle Group. The temperature range of the daily means over the main reef fish recruitment period (October to March) was 25 - 30 °C. These two temperature extremes were chosen for the experiment.

7.4.2 Morphometrics from the temperature experiment

There was no significant difference between the two temperature treatments in any of the three body dimensions measured (i.e. SL, body depths at the pectoral fin and anal fin) (Fig. 7.2). The body depth measurements mirrored trends in the standard length (Fig. 7.2), so only the analysis for the latter is presented (Table 7.1). The mean standard lengths of fish within the 25 °C treatment was slightly larger than those in the 30 °C tanks, and Tukey's tests found that both were significantly smaller than the field sample (Field, Fig. 7.2). However, there were no significant differences between field sample and treatment fish for body depth at the anal fin (Fig. 7.2).

These trends in body dimensions were accentuated in body wet weight, with fish in both temperature treatments being significantly lighter than those which settled the night after capture (Fig. 7.3, Table 7.1). For all three body measurements and weight there were no significant differences among the three tanks within a treatment (Table 7.1).

7.4.3 Age at settlement

The average number of days for a fish to metamorphose and settle to the bottom of the tank differed significantly between treatments (Table 7.1). Fish within the 30 °C tanks settled on average 2.7 days faster than those in the 25 °C tanks (mean 9.24 vs 11.96 days respectively, Fig. 7.4). The time taken to settle did not differ among tanks within a temperature treatment (Table 7.1).

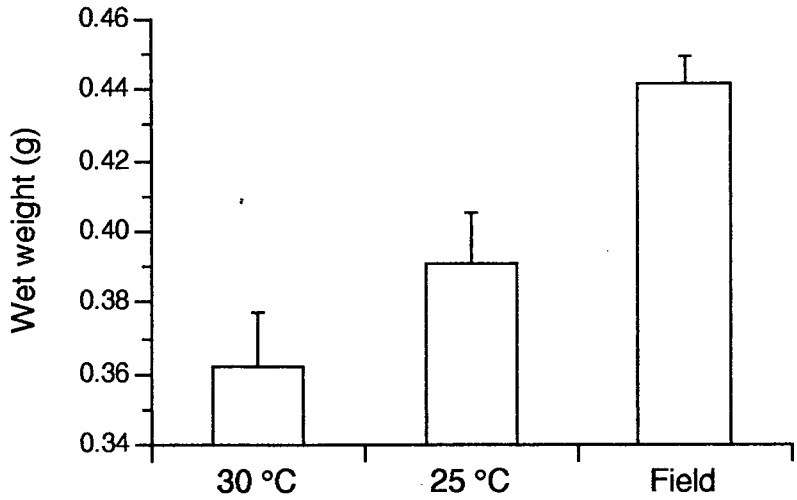

Figure 7.3. Comparison of the mean wet weights of newly settled *Upeneus tragula* among two temperature treatments and a sample of field fish. Error bars are standard errors.

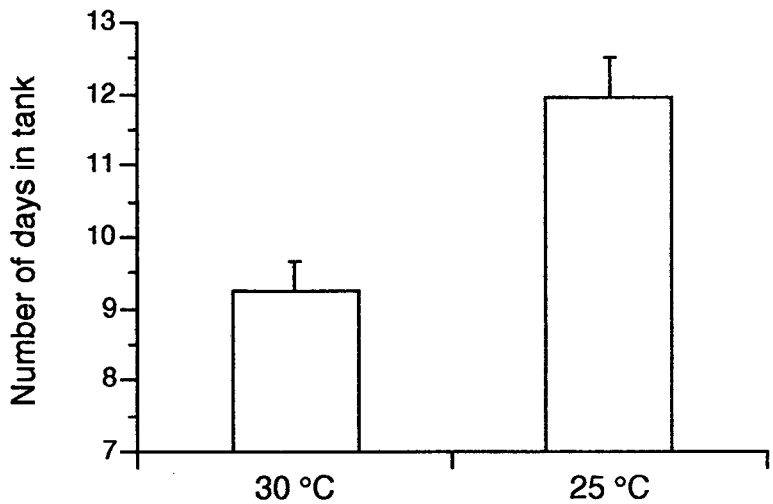

Figure 7.4. Comparison of the average time taken for *Upeneus tragula* to settle in two temperature treatments. Error bars are standard errors.

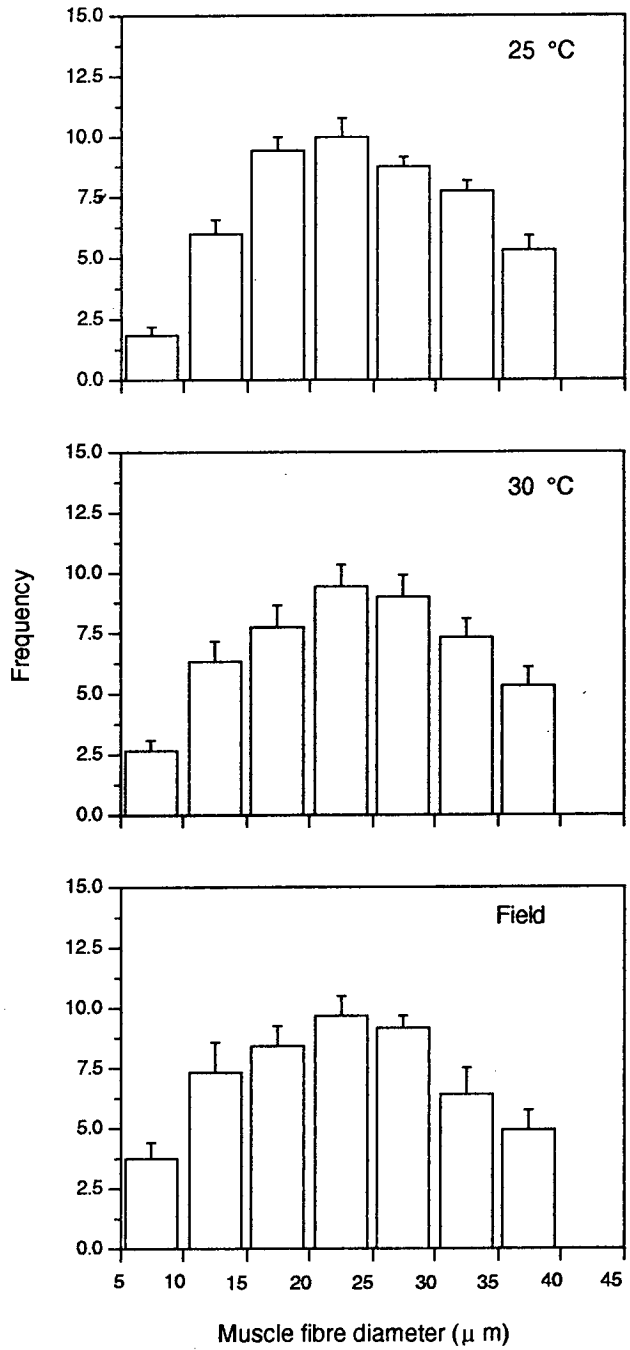

Figure 7.5. Maximum muscle-fibre diameter size frequencies (μm) for *Upeneus tragula* from the field and within the two temperature treatments. Error bars are standard errors (n = 10).

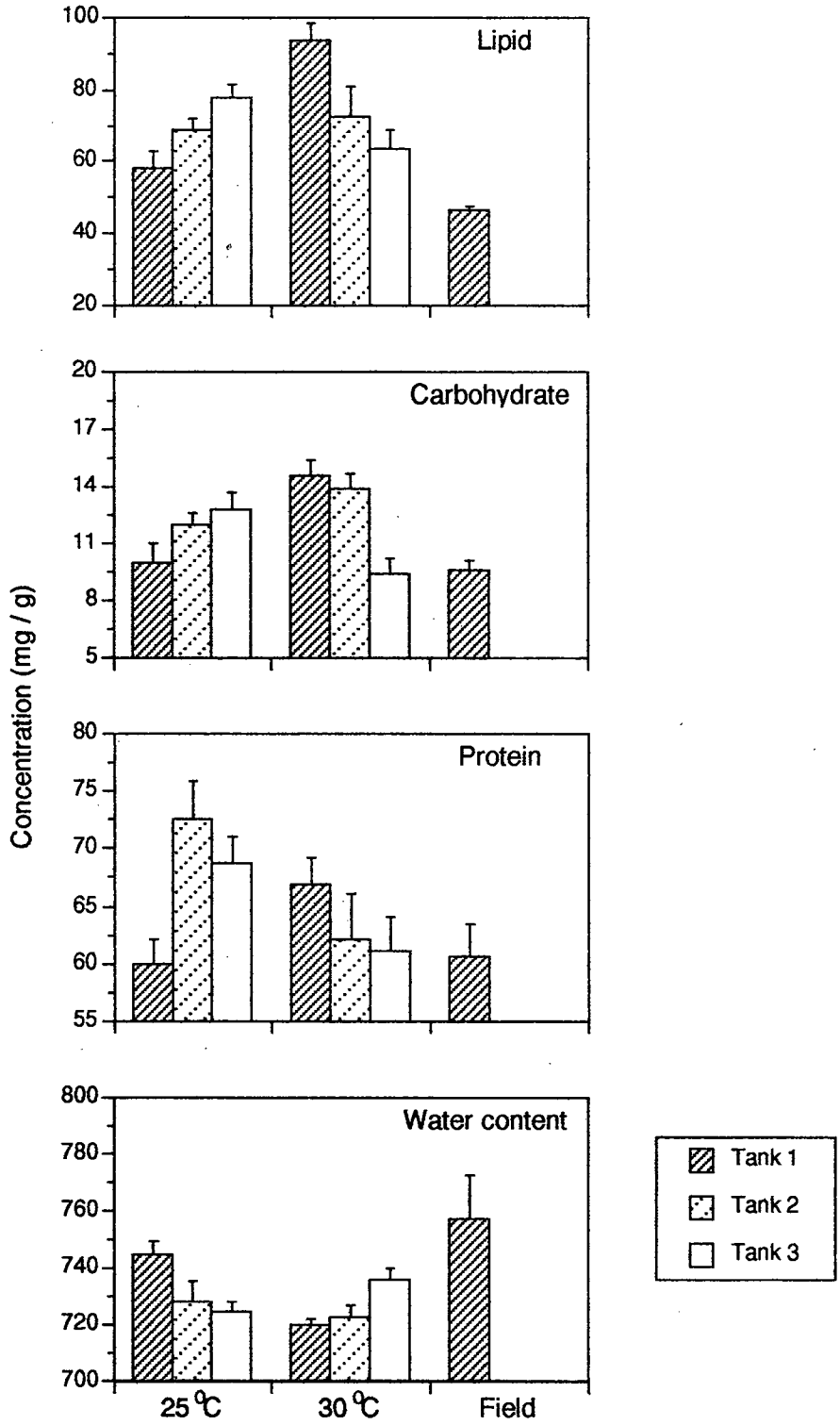

Figure 7.6. *Upeneus tragula*. Comparison of the mean total lipid, carbohydrate , protein and water concentrations (mg / g wet weight) among the field fish and those held within the three tanks at each of two temperatures. Error bars are standard errors.

Table 7.1. Summary of analyses of variance comparing morphological and, biochemical attributes of *Upeneus tragula* held until settlement within replicate tanks, under two temperature treatments. In addition, bite rates of 10 fish within each of 3 tanks per temperature (25, 30 °C) were compared over 3 times through the experimental period (Start, Mid, End, see methods).

Attribute	Source	DF	MS	*p*
Standard length	Temperature	1	11.790	0.2701
(mm)	Tank(Temp.)	4	7.339	0.1685
	Residual	84	4.438	
Weight	Temperature	1	0.0101	0.5140
(grams)	Tank(Temp.)	4	0.0201	0.0935
	Residual	84	0.0097	
Time in tanks	Temperature	1	168.883	0.0084
(days)	Tank(Temp.)	4	7.218	0.6209
	Residual	84	10.921	
Lipid content	Temperature	1	963.300	0.4831
(mg / g wet weight)	Tank(Temp.)	4	1642.936	<0.0001
	Residual	49	222.0128	
Carbohydrate content	Temperature	1	15.162	0.6029
(mg / g wet weight)	Tank(Temp.)	4	48.179	0.0001
	Residual	49	6.0435	
Protein content	Temperature	1	186.390	0.4129
(mg / g wet weight)	Tank(Temp.)	4	225.666	0.0288
	Residual	49	76.260	
Water content	Temperature	1	511.669	0.4827
(mg / g wet weight)	Tank(Temp.)	4	865.067	0.0059
	Residual	49	209.854	
Bite rate	Time	2	240.050	0.0003
(strikes / 15 sec)	Temperature	1	182.005	0.0167
	Time X Temp.	2	18.239	0.2021
	Tank(Temp.)	4	11.611	0.5822
	Time X Tank(Temp.)	8	9.277	0.7997
	Residual	162	16.218	

7.4.4 Muscle development

A maximum likelihood analysis of variance found that there was no significant difference in the maximum muscle-fibre diameter distributions among the two temperature treatments or field sample (Table 7.2, Fig. 7.5). Similarly, there were no significant differences in the muscle size distributions of fish within the tanks, or among tanks within a treatment (Table 7.2).

Table 7.2. Summary of a maximum likelihood analysis of variance comparing the size distributions of muscle fibre diameters from five *Upeneus tragula* within two tanks in three treatments (25 °C, 30 °C, field)

Source	DF	χ^2	*p*
Treatment	12	8.87	0.7142
Tank(Treat.)	18	18.63	0.4151
Fish(Treat. X Tank)	144	114.06	0.9688

7.4.5 Body constituents

There was no significant difference between the two temperature treatments in any of the body constituents measured (Table 7.1). However, there were differences among the three tanks within the treatments for all components (Table 7.1). This was accentuated in the energy storage components (lipid and carbohydrate, Fig. 7.6). Tank 1 at 30 °C showed by far the highest total lipid levels (mean of 94 mg/g wet wt), while fish within tank 1 at 25 °C had the lowest level (mean of 58.3 mg/g). Tukey's tests found that lipid levels of fish within tank 1 at 30 °C differed from all other tanks except tank 3 at 25 °C. The field sample was significantly lower in lipid content than fish from all but the two lowest treatment tanks (tank 1, 25 °C; tank 3, 30 °C).

The 30 °C treatment included tanks with the lowest and highest mean levels of total carbohydrates in the experiment (tank 1, 14.6 mg/g; tank 3, 9.4 mg/g). Tukey's tests found that levels for fish in tank 1 at 30 °C were significantly higher than those within tank 3 at 30 °C, tank 1 at 25 °C, or the field sample (Fig. 7.6). The field sample was significantly lower in carbohydrate than fish in tanks 1 and 2 at 30 °C, and tank 3 at 25 °C.

Total protein content of the fish showed a small although significant difference among tanks within treatments, however, the nature of this

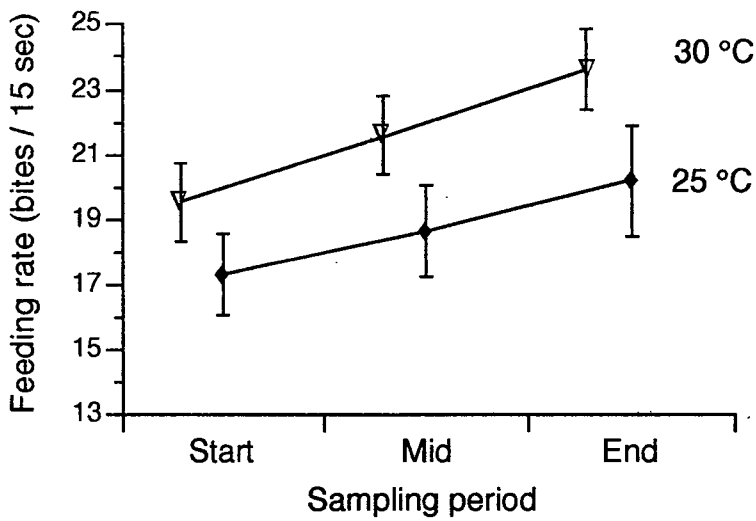

Figure 7.7. *Upeneus tragula*. Mean feeding rates with 95% confidence limits for fish within the two temperature treatments over the experimental period. "Start", represents one day after the start of the experimental period; "Mid", when one-quarter of all the fish within the 3 tanks (for each treatment) had settled; "End", when approximately 10 fish remained in 3 tanks per temperature treatment.

difference was not identified by the *a posteriori* tests. This significant result may have stemmed from the highest and lowest protein levels occurring in the 25 °C treatment (tank 2, 72.5 mg/g; tank 1, 60.0 mg/g). Water content showed the reverse trend to that of the lipid and carbohydrate concentrations (Fig. 7.6). The field sample had the highest levels (757.7 mg/g wet weight), while tank 1 at 30 °C had the lowest (720 mg/g). Tukey's tests found that the field sample had significantly higher water content than fish within tank 1 and 2 at 30 °C or fish within tank 3 at 25 °C (Fig. 7.6).

7.4.6 Feeding rates

Feeding rates of fish within both of the treatments increased significantly over the experimental period (Fig. 7.7, Table 7.1). Average bite rates for fish within the 25 °C tanks were significantly lower than for those in the 30 °C tanks, throughout the experimental period (Table 7.1). There were no differences between tanks within the temperature treatments (Table 7.1).

7.5 DISCUSSION

A 5 °C increase in water temperature decreased the time to metamorphosis by 2.7 days for *Upeneus tragula* reared from midway through their pelagic life phase. This temperature increase did not affect muscle development or the biochemical composition of the fish. However, mean length and body depth were smaller, and mean wet weight lower at the higher rearing temperature, although this was not statistically significant. Interestingly, feeding rate was significantly higher for fish in the warmer treatment. Together, these results suggest that although fish in the warmer treatment were feeding more, this did not result in a larger body mass, but rather was offset by the increase in metabolism at the higher temperature.

Unfortunately, comparative data are few. There are no reliable field data on the effect of temperature on the timing of metamorphosis and settlement in Osteichthyes (Youson 1988), and laboratory studies are limited to the flatfishes. Studies on four flatfishes, the winter flounder *Pseudopleuronectes americanus* (Laurence 1975), the sole *Solea solea* (Fonds 1979), the starry flounder *Platichthys stellatus* (Policansky 1982) and the Japanese flounder *Palalichthys olivaceus* (Seikai *et al.* 1986) show a marked reduction in larval duration with increasing temperature, similar to that found in the present experiment. This dependence of age at

metamorphosis on temperature, suggests that the convention of quantifying these ages by calendar time has little biological value. An alternative measure, suggested by Chambers & Leggett (1992), that incorporates a physiological component is achieved by converting age at metamorphosis to physiological age at metamorphosis, in units of degree-days. Re-scaling time in this way greatly reduces the between population differences in the ages at metamorphosis and highlights interspecific ones. To enable unconfounded comparisons in larval duration among species of reef fish, as has been done for pomacanthids (Thresher & Brothers 1985), labrids (Victor 1986c) and pomacentrids (Wellington & Victor 1989), temperatures experienced by larvae should be reported, even if these are approximated.

In general, the effect of temperature on size at metamorphosis appear slight and species specific. Similarly to the present experiment, studies of *Solea solea* (Fonds 1979), and *Palalichthys olivaceus* (Seikai *et al.* 1986) found size at metamorphosis varied inversely with temperature. However, temperature was directly related to size in *Pseudopleuronectes americanus* (Laurence 1975) and *Platichthys stellatus* (Policansky 1982). Unfortunately, the conclusions from all four studies must be treated cautiously because they were pseudoreplicated (Hurlbert 1984), using only one tank per temperature treatment. This is a serious problem since Chambers & Leggett (1987) found high variability in the size at settlement among 18 tanks of winter flounder from the same spawning, held under similar laboratory conditions. Had the present experiment been initiated at hatching, as in the earlier studies, then the slight increase in size at settlement with lower rearing temperature may have been accentuated. Until further evidence is available we can only speculate whether the difference in the relationship between size and temperature found among studies is taxon specific, an age related response or simply an experimental artefact.

The use of wild caught larvae rather than fish reared from eggs of known parentage reduced the sensitivity of this experiment to changes due to temperature by incorporating genetic and age variability. This will lead to more conservative tests. However, their use has two distinct advantages. Wild caught fish have less tank and rearing artefacts, which Chambers & Leggett (1987) have shown can be high. Secondly, a variable genetic pool and age distribution is a characteristic of the field population. Thus,

conducting an experiment incorporating this variability increases the pertinence of the results to the field situation.

This study suggests that progeny spawned early in the extended reproductive season and developing in cooler water are likely to have slower growth rates, but may settle at a larger size, than those spawned into warmer waters later in the season. This may explain some of the variability in the size at which *Upeneus tragula* is competent to settle. However, the findings of larger recruits earlier in the season are not supported by field observations. Chapter 4 examined the variation in the size of *Upeneus tragula* at settlement over eight samples from a single station off Lizard Island during Nov. and Dec. 1991 (4 samples per month, Table 4.3). Fish that settled early in the season were found to be significantly smaller than fish that settled later (Nov: 26.9, Dec: 28.1 mm SL). Furthermore, the mean age at settlement did not differ between months (32.2, 31.4 d). However, growth rates averaged over the whole larval period were higher in Dec., although this was not statistically significant (0.83 v.s. 0.78 mm / d). This discrepancy between experimental and field observations may be due to an interaction between water temperature and the availability of the planktonic food items on which the pelagic-stages feed. If higher water temperatures are correlated with higher primary productivity then the observation of larger fish settling in the warmer parts of the recruitment season might be expected.

This experiment showed that temperature may also be important in driving the high levels of variability present in the age and growth estimates for a given size at settlement. Changes in larval growth rate have the potential to strongly influence not only the size at settlement of demersal fishes, but also the number of potential settlers (recruitment success). Recently, a number of studies have concluded that small changes in daily growth rates during the early pelagic phase can have dramatic effects on the number of fishes in a year-class (Houde 1987, 1989b, Miller *et al.* 1988, Davis *et al.* 1991, Pepin 1991). Those fish that grow fastest, move rapidly through the range of predatory fields and contribute more to the resulting cohort. Houde (1989a) noted that these subtle changes in growth rates were often as important as episodic mortality due to massive transport loss, disease or environmental perturbations. Thus water temperature may influence recruitment success by modifying growth and developmental rates within the plankton.

However, Buckley (1982) stated that temperature is secondary to food availability in controlling growth rate in larval fish. An extensive body of literature documents the immediate effects of the quantity and quality of food received on the growth rates and body characteristics of larval fishes. Chapter 6 examined the influence of varying food quantity during the late larval stage of the goatfish, *Upeneus tragula*, on the size, weight, muscle development and body chemistry at settlement. Fish were of a similar size to that used in the present study. In contrast to the temperature experiment documented here, major differences were found in all body attributes measured between the feeding treatments. For instance, I found highly significant differences at settlement in total lipid, carbohydrate and protein content of fishes that had been under differing feeding regimes during their early pelagic life. However, the present study found no coherent differences in body composition between the two temperature treatments, with any differences being sufficiently small to be swamped by variation among fish within tanks. This is despite the fact that the range of the body constituent levels among tanks within treatments was lower in the temperature experiment than in the feeding experiment (i.e. the temperature experiment had a more sensitive test). At least for the pelagic life stage studied here, food availability appears to have a greater influence on fish size and development than water temperature.

Little is known of the relative importance of food and temperature during the earlier developmental stages. Prior to first feeding, exogenous food availability is unimportant and the abiotic factors of temperature, oxygen partial pressure and salinity have a greater potential to influence the foundations on which subsequent development will build. Temperature may also affect larval growth potential prior to spawning (Buckley *et al.* 1990). Many of the relative effects of varying food availability (e.g. differences in weight, muscle development), apart from systems degeneration caused by complete starvation, have been found to be reversible once normal conditions were restored. Weatherley & Gill (1981) studied the effect of 16 weeks of limited rations, and of starvation for 3 and 13 weeks, on the weights and tissue mass of immature rainbow trout. They concluded that non-degenerative periods of starvation offset growth but had little permanent effect on the growth potential of the fish. Similarly, in Chapter 6 I experimentally starved late stage larval *Upeneus tragula* for a three day period and then re-fed *ad libitum* until settlement. When the growth attributes of these fish were compared to fully-fed fish, I found that

the perturbed fish differed little in growth characteristics and settled only three days after the fully-fed treatment. This suggests that by midway through their pelagic life history *U. tragula* are resilient to short periods of starvation. Consequently, I suggest that temperature has the potential to have a greater *permanent* effect on the growth potential and form of fishes than sub-lethal food availability due to its influence during the sensitive differentiation stage.

When taken in concert, the feeding experiment (Chapter 6) and the present experiment suggests a dissociation of the processes of growth and differentiation for the late pelagic-stage fish under certain environmental conditions. Temperature did not uncouple differentiation and growth rates within the limits used in this experiment. Fish within the 30 °C treatment not only had a faster growth rate, but also a faster developmental rate than those within the 25 °C tanks; further, fish did not significantly differ in their size at settlement. On the other hand, food availability did cause dissociation. Food not only changed the growth rate to settlement but also the size at metamorphosis. Fish fed *ad libitum* settled at 29.0 mm SL after 14 tank days, compared to fish fed once-per-day that settled at 25.9 mm SL after 23 days. Susceptibility to dissociation may change ontogenetically, and for *Upeneus tragula* the 5 °C temperature range may not elicit a response at the late larval stage used in the experiment (starting approximately half-way through the pelagic phase). Different stages of salmon embryogenesis, some dominated by growth and others by organ development, have very different temperature coefficients (Trifinova *et al.* 1939: cited in Lindsey 1988). Pertinent models for the relationship between growth and differentiation to metamorphosis have been proposed by both fish and anuran researchers (e.g. Youson 1988; Lindsey 1988 for fish; Wilbur & Collins 1973, Travis 1984, Alford & Harris 1988 for anurans). However, further information is required for reef fishes before the applicability of these models can be assessed.

This study suggest that the size, age and body condition of *Upeneus tragula* at settlement is a product of the combined and interactive effects of water temperature and food availability on the fishes' growth and developmental rates. Sensitivity to temperature or nutritional fluctuations is likely to change with ontogeny. During embryogenesis and development to yolk sac absorption, water temperature is important in determining the fishes' potential for subsequent growth and development (e.g. Lindsey &

Harrington 1972, Fonds *et al.* 1974, Harrington & Crossman 1976). However, during the pelagic stage food availability appears to have more effect on the body characteristics of the settling fish than does water temperature. Future research should manipulate the earlier life-history stages to determine the interactive nature of temperature and food on the developmental state of larval fish through to settlement. Serial sampling of such a temperature and food experiment will determine the relative importance of the two factors and their interaction at particular life stages. This will aid in the development of a model for the growth and differentiation of reef fishes.

Chapter 8

GENERAL DISCUSSION

8.1 MAJOR FINDINGS

The present study highlights the potential importance of planktonic processes and events in influencing demersal life stages of coral reef fish. The study has focused on the variability in the quality of fish when they first enter the reef system. This variability may influence the numbers of settlers that survive to be counted in recruitment surveys, and which individuals reach the juvenile and subsequently the reproductive population. This is a novel approach for two reasons. Firstly, it has been logistically difficult to link the pelagic and demersal phases of the life cycle. Secondly, most studies have focused on the magnitude and timing of replenishment (reviewed by Doherty & Williams 1988, Doherty 1991). By concentrating on quality rather than quantity, this approach does not displace a numerical perspective but provides an improved focus and aids in explaining what drives numerical change. The integrated perspective gained by examining details of the pelagic ecology, metamorphosis and settlement transition of goatfish has revealed some novel insights into how the pelagic phase interacts with the fishes' developmental physiology to influence the survival potential of newly settling reef fish.

The conclusion that larval reef fish are far from the passive plankters once assumed by the literature is now commonplace (e.g. Kingsford 1988, Leis 1991b, Milicich 1992, Choat *et al.* In press). This statement is based on the size of the late pelagic stages and their apparent sensory competence, rather than direct measures of physical ability or known active dispersal patterns. Preliminary information on sustained swimming speed (Chapter 2) and detailed data on burst swimming speed (Chapter 5) are the first indications that late pelagic-stage reef fish exceed present expectations of locomotory ability.

A recently completed study of the sustained swimming speeds of a variety of reef fishes further emphasises the potential for active dispersal over long distances. Stobutzki (1992) placed a single recently settled *Upeneus tragula* (29.2 mm SL) in a flume chamber and increased the current speed by 5 cm/s every 10 min. She found that the fish could swim in excess of 44.4 cm/s (15.2 body lengths / s); well above the maximum

current speed of 38 cm/s measured at five stations across the northern GBR. However, to actively disperse large distances swimming must be coordinated in a particular direction, rather than simply haphazard orientations associated with feeding. If schools of larvae cruise haphazardly, then their net displacement from their spawning location will largely be hydrodynamically driven. In this circumstance passive drift models will be useful in predicting the dispersal of larvae from a source reef (e.g. Black & Gay 1987, Black *et al.* 1991). The extent to which dispersal is active can only be determined by tracking larvae (tagged genetically or with a chemical tracer) from a source reef to their settlement destinations through waters of known hydrology.

Sampling by aggregation devices enabled the collection of the whole size range of pelagic stage fish, from early in their pelagic life through to competence to settle. This revealed a complex school organisation that defies conventional theories of the hydrodynamic advantages of school formation (e.g. Shaw 1970, Lindsey 1978). These schools incorporated a number of goatfish species (at times four or more), each with a broad size distribution. Much of the variation in energy storage compounds (carbohydrates and lipids) found for newly settled *Upeneus tragula* was explained by differences among samples, each of which may represent part of a single school (71 and 53 % of the total variation respectively). This suggest that these schools have a high degree of temporal persistence.

Evidence suggests that much of the variability in size and age of *Upeneus tragula* at settlement is explained by the environmental and biotic conditions experienced by these pelagic schools, that directly influence the growth and differentiation rates. Despite statements to the contrary (Victor 1986b, Cowen 1991, Kaufman *et al.* 1992), there is presently little evidence for extensive delays of metamorphosis through active choice for any reef fish species. The feeding experiment presented here suggests that the feeding history of a fish during its larval phase is a major determinant of the growth attributes at settlement. Further, food availability and, to a lesser extent, water temperature had a major influence on when a fish was competent to settle. The degree to which the settlement event can be delayed due to an absence of appropriate settlement cues is yet to be addressed. It appears that the availability of planktonic prey and water temperature may play major roles in determining the limits to larval

dispersal through their influence on developmental rates and condition, and ultimately larval duration. Thus the way a particular species physiologically responds to conditions in the plankton may govern the persistence and genetic integrity of widely distanced populations that are only replenished by propagules from distant reefs.

The feeding experiment suggested that *Upeneus tragula* were physiologically well suited to using a patchy food source in the larval stage. Larvae that were starved for three days and then re-fed settled with similar body attributes (size, muscle development and biochemical condition) to those that had been fully fed. This suggests that a wide range of feeding histories, involving intermittent periods of low and high food intake, may lead to the same conditional endpoint. However, for all fish species, the rate of feeding and the efficiency of consumption of food, and how this relates to fish size, may be very different depending on whether the fish were deprived of food at any stage during their life history (Talbot 1985). A comparison of field fish with fish from the four feeding treatments suggests that the normal feeding intensity is considerably less than *ad libitum*. In less than *ad libitum* feeding conditions differences in utilisation rates caused by individual feeding histories may account for some of the high variability in body composition among newly settled individuals within a sample.

Food availability strongly influenced development of the somatic system, but had little effect on the sensory system. There appears to be a trade-off between development of sensory system and the length of larval duration. Fish in the fully fed treatment settled younger, larger, in better biochemical condition and with more highly developed muscles than less well fed fish. However, these fish also had the least developed barbels. Interestingly, prior to metamorphosis in *Upeneus tragula* there was a considerable range in the state of sensory development, as measured by the distance of the hyoid arch (to which the barbels are attached) to the tip of the dentary. By the time settlement had occurred 6 - 12 h later, the hyoid arch abutted the dentary in all individuals. This suggests that metamorphosis is more dramatic in some fish than in others, but more importantly, that this aspect of sensory development was not directly related to the timing of metamorphosis. Furthermore, larval *U. tragula* that had been starved for 6 days, when over 50% mortality occurred per day, still metamorphosed. Thus it appears that somatic and sensory developments are independently controlled and although environmental conditions have

an influence on when metamorphosis occurs, they do not appear to directly control it.

So what does control metamorphosis? It has been experimentally demonstrated that metamorphosis in flatfish (e.g. De Jesus *et al.* 1991, Yamano *et al.* 1991) and the smolt transformation in salmonids (e.g. Hoar 1988, Redding *et al.* 1991) is controlled by levels of the developmental hormones thyroxine (T_4) and triiodothyroxine (T_3), in conjunction with the steroid cortisol (De Jesus *et al.* 1990, Redding *et al.* 1991). However, it is currently unknown how production of the hormone is influenced by the fishes' physiology, which will be directly influenced by the environment (salinity, temperature, nutrition, e.g. Grau 1988) and behavioural responses of the fish. The way that the magnitude of the variability in age at settlement differs among species (e.g. Thresher & Brothers 1989, Victor 1991, In press) suggests that, although the hormonal mechanism underlying development may be taxon-wide, how environmental variables interact with the fishes' physiology to produce a developmental response may be species specific.

Although the details of the changes in the gustatory and visual system that occur at metamorphosis may be specific to mullids, changes of this speed and magnitude may not be atypical in reef fishes. Macroscopic changes that occur during metamorphosis in mullids simply involve changes to body pigmentation. The silver pelagic colouration changes to a mottled cream and fin and body stripes appear. Such changes are commonplace amongst reef fish. Dramatic changes to the shape of the fish, that occur in surgeonfish (Randall 1961) and serranids (Leis & Rennis 1983), do not occur in mullids. As researchers undertake detailed investigations of metamorphosis, changes in physiology and internal structure will be found to compliment subtle or major changes in body shape, marked changes in pigmentation and loss of spination. These studies will accentuated the differences rather than the similarities between pre- and post-settlement fish structure and their ecologies.

Measuring the condition or quality of a newly settled reef fish was found to be a difficult task. No single measure of body form, composition or performance adequately described the potential of an individual to survive the structuring processes of predation, competition and starvation. This was complicated by high levels of variability in all measures and a lack of correlation among them. This suggests that measures of condition should

be used cautiously. It is generally assumed that indices of condition relate to the fishes' ability to survive in their environment. However, it is likely that the relationship between an index of condition and survival potential will vary between habitats and times. For instance, Shulman (1985), in an experimental study, found that the risk of predation for juvenile reef fishes decreased with distance away from the reef front. Under these circumstances, a particular level of stamina and burst speed for a prey species that may be adequate at the back reef may be insufficient to avoid the higher predation pressure at the front. The high variability in body condition demonstrated for *Upeneus tragula* begs the question of its importance to the reef population. How are the levels of variability in quality found among newly settled fish modified by post-settlement events? Does the "quality" of a fish bias the probability of it surviving to maturity or its level of fecundity once mature?

8.2 IS FISH QUALITY IMPORTANT AT SETTLEMENT ?

Mortality of newly settled fish is highest during the first days or weeks after settlement (Doherty & Sale 1985, Shulman & Ogden 1987, Meekan 1988, Sale & Ferrell 1988, Hixon 1991, Jones 1991). Predation has been suggested as the main cause of this mortality, with at least 25% of newly settled fish dying in the first five days on the reef (Doherty & Sale 1985, Aldenhoven 1986, Victor 1986a, Eckert 1987). An estimated 8 to 53% of reef associated fish feed on newly settled reef fish (Hixon 1991). However, due to the rapid attack speed, and the transitory and unpredictable nature of predation episodes, the role of predation is assumed rather than demonstrated. Given the high variability in all aspects of fish condition at settlement it is important to know how the initially high mortality alters the variability in fish condition. A knowledge of the vulnerability to predation in early post-settlement life is central to any attempt to understand the basis of year class strength and recruitment to the reproductive population.

Laboratory and field studies are required to test hypotheses regarding the influence of quality on the survival potential of reef fish. Two examples of such an approach are included in Appendix 2. These experiments test two aspects of the conventional size-based theories of survival (i.e. "bigger-is-better", Miller *et al.* 1988, Litvak & Leggett 1992). Firstly, those fish that are large will have a greater probability of escaping predation than smaller individuals. Secondly, fish of higher biochemical condition will be better able to escape predation. Both found that predation

by the ambush predator, *Synodus variegatus* (lizardfish), was random with respect to both size and biochemical composition of the newly settled fish. This research is ongoing and further results are required before definitive statements can be made. When combined with extensive field observations, this approach has the potential to make substantial advances in our understanding of the mechanism of predation and how it influences replenishment.

If the experiments are indicative of the true nature of predation, that it is actually a random process, then this has important ramifications for the dynamics of recruitment. If predation operates randomly then the levels of variability in growth and behavioural attributes of newly settled fish will have a chance to be accentuated by post-settlement processes such as competition. This variability is likely to be most rapidly accentuated where recruits form small social groups, such as in many of the pomacentrids (e.g *Dascyllus aruanus*, Forrester 1990). Under this scenario it is of interest to know whether it is the individuals that were in "best" condition (measured by whichever index is most appropriate) when they settled that are subsequently high in the dominance hierarchy. If this is the case then the pelagic life history has a strong influence on events in the settled population.

The alternative hypothesis is that predation on newly settled reef fish acts as a bottle-neck on the variability in body condition. Convention suggests those individuals that are small and in lowest body condition may be consumed in preference to larger more able settlers (e.g. Miller *et al.* 1988). However, given the poor correlations found among a range of different estimates of body condition (Chapter 5), if predation is targeted on one particular trait (e.g. body length), then it will not reduce the variability in other aspects of body condition (e.g. growth rates, biochemical composition). Under this scenario the influence of predation on the structure of a recruit cohort, mediated through a particular aspect of quality, will depend upon the combination of features of quality that are most important for success in a particular situation. For example, predation with respect to fish size will have a major effect on a population of clown fish, *Amphiprion clarkii*, where a size structured hierarchy governs growth and reproductive status (Moyer 1980). Predation with respect to condition factor may, however, have little influence on the status of individuals in the social group.

Recently, two studies have found evidence contrary to the "bigger-is-better hypothesis" (Litvak & Leggett 1992, Pepin *et al.* 1992). Both examined the effects of predation by sticklebacks, *Gasterosteus aculeatus*, on yolk-sac capelin larvae, *Mallotus villosus*, by introducing two size classes of larvae into a mesocosm and examining the survivors after a period of predation. Both studies found that, despite the larger larvae being less vulnerable to attack due to their higher mobility, they sustained higher mortality when predators could choose among larvae of varying size (4 - 6.2 mm SL). To further complicate matters, Webb (1982), found that the attack strategy of the predator and its body form contributed greatly to the response of the minnow prey (*Pimephales promelas*) to four different predators. Prey responded to only 28% of strikes by the slender, bullet-shaped esocid (*Esox*), while responded to 75-90% of strikes by the three more laterally compressed predators (trout, bass, rock bass). These and other studies (e.g. Yin & Blaxter 1987, Fuiman 1989, Margulies 1989) demonstrate that there is a hierarchy of processes that determine the probability that a fish will be preyed upon. Studies such as these have laid the foundations for understanding the importance of size and competence of the prey in predator encounters for the early larval stages of some marine fish. Obviously for reef fishes, there is a long way to go before we can understand how predation influences the distribution of juvenile quality, and how these interact to influence patterns of distribution in reef fish populations.

8.3 CONCLUSION

The contribution of pelagic processes to the settled population is more than just numerical. The interaction of the larval ecology of the fish with conditions in the pelagic result in individuals settling into the reef population that are of variable growth rate, energy stores, swimming speed and overall quality. Moreover, these attributes vary significantly over a range of temporal and spatial scales. For the goatfish, *Upeneus tragula*, much of this variation appears to be driven by the interaction of larval schools with a food source that is patchy, in both quality and availability. The extent to which genetics, parental input and density dependence modify the growth attributes of larvae within a school is presently unknown. The variability found in the quality of newly settled reef fish may have a major influence on which individuals survive to the reproductive population and their abundance patterns. When linked with estimates of numerical

abundance, qualitative data has the potential to greatly advance our understanding of the mechanisms driving replenishment of reef fish populations.

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Appendix 1

Publications resulting from this thesis

This is a summary of the publications that have to date resulted from this study. Copies of papers published or in press are included in this section.

Published:

McCormick, M. I., Molony, B. W. (1992). Effects of feeding history on the growth characteristics of a reef fish at settlement. Mar. Biol. 114: 165-173

In Press:

McCormick, M. I., Shand, J. (In press). Metamorphosis of the visual and barbel sensory systems at settlement in the reef fish *Upeneus tragula* (Family Mullidae). Proc. 7th Int. Coral Reefs Symp., Guam.

McCormick, M. I., (In press). Development and change at settlement in the barbel structure of the reef fish, *Upeneus tragula* (Family: Mullidae). Environ. Biol. Fish.

McCormick, M. I., Molony, B. W. (In press). Quality of the tropical reef fish, *Upeneus tragula* (family: Mullidae), at settlement: Is size a good indicator of condition? Mar. Ecol. Prog. Ser.

Submitted:

McCormick, M. I., Molony, B. W. (submitted). Effects of temperature during the pelagic life stage on the growth characteristics of a reef fish at settlement. J. Exp. Mar. Biol. Ecol. (submitted Aug. 1992)

McCormick, M. I. (submitted). Variability in age and size at settlement in reef fish: how much is there and what can it tell us about the process of settlement? Mar. Ecol. Prog. Ser. (submitted Nov. 1992)

McCormick, M. I., Milicich, M. J. (submitted). Late pelagic-stage goatfishes: distribution patterns and inferences on schooling behaviour. J. Exp. Mar. Biol. Ecol. (submitted Dec. 1992)

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Effects of feeding history on the growth characteristics of a reef fish at settlement

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Metamorphosis of the visual and barbel sensory systems at settlement in the reef fish Upeneus tragula (Family Mullidae)

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Abstract. Settlement for a reef fish involves a major change in sensory stimuli as the fish undergoes metamorphosis and moves from a planktonic to demersal existence. This study examines the nature of structural changes in the sensory system at settlement for the goatfish Upeneus tragula (Family Mullidae). Pelagic-stage fish were collected using purse-seines around aggregation rafts or in light-traps off Lizard Island, on the northern Great Barrier Reef, during the summers of 1989/90 and 1990/91. Settled fish were caught from the reef using fence-nets. Changes in the visual system and outgrowths of the olfactory and gustatory system (barbels) are examined in detail, using various morphometric and histological techniques. Major changes were found to occur in both systems. An unusual double-layer of cone photoreceptors and high densities of bipolar (neural processing) cells were found in the dorsal retina during the pelagic stages; this double-cone layer was lost, and both the cone and bipolar cell densities reduced during the 6 - 12 h settlement period. This may represent an overall reduction in relative visual acuity. Sensory barbels, which aid in the detection and manipulation of prey items once settled, underwent rapid growth at settlement, largely due to the expansion of taste bud cells. These changes to the sensory system are likely to be linked to the changes in environmental stimuli which occur during settlement.

Introduction

For reef fish, the transition between the pelagic larva and the reef-associated juvenile may be of crucial importance in determining the abundance patterns of fish on the reef (Doherty 1992). This settlement process represents the greatest change in the suite of environmental and biological pressures that a reef fish will actively experience during its lifetime (McCormick and Molony In press). Factors such as light regimes, turbidity, water chemistry, prey types and availability, and predator pressure are likely to differ greatly between the two environments. Structural changes which occur during the metamorphosis associated with reef-fish settlement, will interact with the existing functional capabilities of the fish to determine its response to these new sensory cues, and hence the fish's ability to capture prey and escape predators.

The few studies done to date suggest that structural and functional changes which occur at settlement are varied, and range from subtle changes in pigmentation (some Pomacentrids e.g. Abudefduf sp., McCormick unpublished data) to major reorganisations of the physiology, somatic and sensory systems of the fish (e.g. Pleuronectiformes, Laurence 1975, Neave 1986). The potential for high mortality at settlement and the nature of the structural, physiological and behavioural changes which occur at metamorphosis have led some researchers to refer to settlement as a "critical period" (Blaxter 1988, Browman 1989).

The present study examines the changes which occur in the sensory system of the tropical goatfish, Upeneus tragula (Family Mullidae), as it undergoes metamorphosis and settles into the reef environment. As for many reef fish (Sweatman 1985a), settlement in U. tragula is a rapid, often overnight process. In this species it constitutes a shift from a pelagic existence as an active predator in the top 6 m of the water column (Leis 1991, McCormick and Milicich submitted) to becoming a benthic microcarnivore which is closely associated with the substratum. Furthermore, the pelagic stages rely largely on vision for the capture of prey items, while the juvenile stages use projections of the olfactory and gustatory systems, in the form of sensory barbels, to detect and manipulate prey. This study looks at the changes which occur in these two sensory systems during metamorphosis against the framework of ontogenetic development. This study represents a summary of the preliminary results of two collaborative studies, the details of which are documented elsewhere (McCormick submitted, Shand submitted).

Materials and methods

Study species

The freckled goatfish, Upeneus tragula (Richardson), is a common component of the ichthyofauna of the central and northern Great Barrier Reef lagoon. Early life stages between 8 mm standard length (SL) and settlement size (19 - 34 mm SL) form large schools with other pelagic goatfish species, and will actively aggregate under drifting debris (McCormick and Milicich submitted). Settlement involves an obvious change in pigmentation and behaviour. The silvery ventral and lateral surfaces present in the pelagic-stage fish change to a mottled cream and brown colouration; dark bars on the caudal and dorsal fins, and a dark stripe from the front of the snout to the caudal peduncle develops.

Collection

Pelagic stage Upeneus tragula were collected from 0.8 to 5 km west of Lizard Island (14°41'S, 145°27'E) on the northern Great Barrier Reef, over approximately 22 m of water during the summer of 1989/90 and 1990/91. Fishes were collected in two ways: some were attracted to a series of 1 x 1 m plastic aggregation rafts moored for 2 h, and were caught with a 14 x 2 m plankton-mesh purse-seine. Others were caught using automated light-traps (Doherty 1987) moored approximately 0.8 km off the fringing reef. Fish from the whole size range available were either placed into a preservative (details below) immediately upon capture, or returned to the laboratory alive and placed into an aquarium. Overnight, some of these fish metamorphosed, changed pigmentation and settled to the bottom of the tank. These fish are regarded as having been competent to settle at the time of capture. Other fish had started to attain the characteristic reef-colouration but had not completely metamorphosed and settled. Individuals in fully- and partially-settled states were preserved for histological examination. Juvenile U. tragula were collected using a fence-net from the seagrass beds within Watson's Bay, on the back-reef of Lizard Island, and preserved for comparison to newly-settled fish.

Histological Preparation

Visual system: A size range of Upeneus tragula that were in their pelagic phase (purse-seine and light-trap samples) and newly-settled phase (fence-net samples) were preserved upon capture. The eyes were pierced and the whole animal was fixed in marine Bouins for 24 - 48 h. Specimens were then flushed a number of times in 70% ethanol and stored in this

preservative for approximately 4 weeks before processing. Partially and newly aquarium-settled fish were similarly preserved. Histological preparation for light microscopy involved dehydrating the heads of the fishes in an alcohol series, treating them in 0.5% low viscosity nitrocellulose (LVN) in methyl salicylate (MS) for 12 h, then infiltrating them with low viscosity nitrocellulose. Lastly, the fish eyes were infiltrated and embedded in parafin wax. Radial serial sections (3 - 4 µm) of one eye per fish were cut and stained in haemotoxin and eosin.

Counts of the number of cells were made in each of the following layers from the radial section: cone inner segments (cones), external nuclear layer (rod and cone nuclei) and bipolar (processing) cell-layer (Fig. 1). Counts were made with an ocular graticule over a strip of the retina 52 µm wide. Ten counts were made in the dorsal and ventral retina of each fish. After correction for section thickness (Abercrombie 1946), the density of each cell type was calculated (per 0.01 mm^2).

Barbels: Cross-sections (6 µm) of the barbels were cut one-third of the total barbel length from the tip, and stained with Azan. Scanning EM had previously shown that taste bud cells were largest and in highest densities in this region of the barbel (McCormick submitted). The area of the epidermis, taste buds, connective and neural tissue, muscle blocks and cartilage were quantified directly from the histological slides using a calibrated digitizing computer package. Density of taste buds per millimetre circumference was also determined. Taste bud cells were recorded as being present in a particular section only if they possessed both a neural connection to the main neural plexus of the barbel and the microtubules penetrated the outer squamous epidermis through a taste pore (Fig. 1b).

Results

Visual system

Examination of the histological sections showed that Upeneus tragula has a duplex ventral retina, typical of diurnal teleosts, and the cell-layer arrangement of this half of the retina was not found to change during development. In contrast, the development of the dorsal retina involved marked changes in the structure of the cell layers. Pelagic stage fish below 17.5 mm SL were found to have an arrangement of cells similar to that in the ventral retina, but between this size and the size at which metamorphosis occurred (in this study ~28 mm SL), the dorsal retina developed a double-layer of cones, as opposed to the single layer found

previously (Fig. 1a). This double-layer is initially confined to the retinal margins, but extends to cover the entire dorsal retina prior to metamorphosis. During the 6 - 12 h period over which metamorphosis occurred in these fish, the structure of the dorsal retina reverted back to the cell-layer morphology of the ventral retina, with only a single layer of cone cells.

Densities of the cone cells, the receptors responsible for diurnal acuity and colour vision, decreased in density as fish length increased in both the ventral and dorsal areas of the retina. The pattern of decline was similar in both areas with an abrupt decline at settlement which was most pronounced in the dorsal retina. The results for the dorsal retina are shown in Fig. 2). A comparison of the densities between partially- and newly-settled fish suggests that the rate of decrease in the cones cells maybe in the order of 25% during metamorphosis (Fig. 2).

The processing efficiency of the retina can be gauged by the number of receptor cells (cones and rods) which input information to the processing neural cells (horizontal, bipolar and amacrine cells) (Pankhurst 1989). The ratio of the photoreceptor to bipolar cells was low prior to settlement when, in both parts of the retina, there was an increase in the number of photoreceptors which are converging to each bipolar cell (Fig. 3). This trend was accentuated in the ventral retina. Calculations of theoretical visual acuity were made using cone density and lens diameter data following the formula given by Tamura and Wisbey (1963). This gives an indication of the ability of the eye to resolve fine detail by calculating a minimum separable angle in minutes of arc; thus the smaller the angle the better the acuity. For Upeneus tragula acuity increased during larval growth to an asymptote of 26 min, which corresponded to metamorphosis.

Barbel development

Mid-way through the planktonic phase of Upeneus tragula (8 to 9 mm SL), barbels develop as thickenings along the edge of the branchiostegal membrane, originating from the hyoid complex (Leis and Rennis 1983, McCormick pers. obs.). The standard length the fish had attained when the barbels detached from the branchiostegal rays was found to be highly variable, ranging from 10 to 22 mm SL. Barbels ranged in size from 0.9 to 2 mm when first detached. During the pelagic life-history phase barbel growth was linearly related to fish length (Fig. 4, Barbel length = 0.194 SL - 1.554, r^2 = 0.852, n = 159). Barbels rapidly increased in length during the metamorphosis associated with the settlement (Fig. 4); a process which in

fish that had been brought into the laboratory from the field, took 6 - 12 h. This was a growth spurt which may represent an increase in barbel length of up to 52 % in 12 h. Upon settlement, barbel growth returned to approximately the same rate as prior to metamorphosis (Barbel length = 0.163 SL + 2.298, $r^2 = 0.223$, n = 12).

The cross-sectional area of the barbels showed a rapid, although less dramatic, increase during the settlement period (Fig. 5). Pre-settlement stages exhibited an exponential rate of increasing area. This was due to the rapid proliferation and differentiation of the sensory taste bud complexes, which form a major component of the epidermis. The epidermal component of the cross-sectional area increased linearly to 70% just prior to metamorphosis, increased approximately 5% during settlement, and then fluctuated around 70% during the juvenile stage (Fig. 6). This rapid increase in the epidermal area was due to a rapid increase in the size of the taste-buds within the epidermal layer. After settlement other components of the barbels, such as the neural bundle, nerve and connective tissue layer (dermis) and cartilaginous supporting rod, offset the increase in taste-bud size, thereby maintaining the epidermal contribution at about 70% of the cross-sectional area (Fig. 6).

Discussion

In many cases there exists a clear correlation between the ontogenetic timing of structural and functional changes in the sensory systems of developing fishes and changes in behaviour, which in turn coincide with ontogenetic shifts in their ecology (e.g. Blaxter 1975, Ahlbert 1976, Noakes 1978, Noakes and Godin 1988). This study is an example of the rapid changes which can occur in the visual and gustatory systems of a fish during the metamorphosis associated with settlement into the reef population. The sensory morphologies of the pelagic and demersal life-history phases are well suited to the environments in which they must efficiently attain food and survive.

Larval and pelagic juvenile stages of Upeneus tragula are clupeoid in appearance and form large schools in the top 6 m of the water column during daylight hours (Leis 1991). They are planktonic predators and field observations suggest that they use a combination of visual and lateral line senses to detect prey and maintain position within the school. It may be that the high cone densities and low convergence ratios maintained in the retina during the pelagic phase are to increase capability for the visual demands of a planktivorous habit. Visual acuity in small eyes is inherently

restricted by lens size. Thus as the eyes of larval fish grow there are dramatic improvements in acuity due to growth of the lens alone. A second factor affecting acuity is the density of the photoreceptors, a situation analogous to the effects of grain size on photographic film. In Upeneus tragula a high cone density is maintained prior to settlement which implies that the high acuity is of significance when in the pelagic phase. A number of studies of pelagic and planktivorous fish have found high cone densities associated with the visual search axis (e.g. Tamura 1957, Tamura and Wisbey 1963, Browman et al. 1990). The acuity values calculated for pre-settlement U. tragula are similar to those found during the growth of the planktivorous damselfish Pomacentrus moluccensis (Shand unpublished data). However, at settlement in U. tragula , acuity no longer continues to improve, whereas in P. moluccensis there is a further increase in acuity to a minimum separable angle of about 14 min. The acuity calculated for adult pelagic species of, for example, the skipjack tuna (Tamura and Wisbey 1963) is even greater at 2.3 min. Low ratios of the convergence of cones to neural cells as found in pre-settlement U. tragula will also help maintain high acuity at this stage of their lifecycle. The processing of information received by the photoreceptors begins in the neural cell-layers of the retina and there are differing degrees of summation in species occupying different reef habitats (Pankhurst 1989). A high convergence ratio would increase the sensitivity of a retina but at the expense of acuity (Lythgoe 1979).

The pelagic environment is rich in ultra-violet and upwelling blue light, while after settlement to the reef substratum the blue component will be replaced by longer wavelength reflections from the sand and algae (McFarland and Munz 1975). The displacement of cones into two layers in the dorsal retina of pre-settlement Upeneus tragula may be a mechanism to overcome chromatic aberrations caused by short wavelength light being refracted by the spherical lens more than long wavelengths (see Fernald 1988). The reorganisation of the cone double-layer into a single layer may be associated with a change in the spectral sensitivity of the receptors, concomitant with the change in the spectral qualities of the light environment at settlement. Indeed it has recently been found that loss of ultraviolet sensitivity during ontogenetic habitat changes in the brown trout (Salmo trutta) and yellow perch (Perca flavescens) is associated with loss of small single cones from the retina (Bowmaker and Kunz 1987, Loew and Wahl 1991). Preliminary measurements of spectral sensitivity of the cones of U. tragula indicate that there are changes occurring at the time of settlement (Shand unpublished data).

Concomitant with this marked change in the visual system, the present study found complementary changes to the barbel microstructure. The length of the barbels increased dramatically during settlement, largely through the rapid expansion of the sensory taste-buds. These complexes comprise about 37% of the total cross-sectional area of the barbel (50% of the epidermis) at this stage. To stress the potential importance of these changes it is necessary to understand the form and function of the barbels in goatfish. Barbels are modified extensions of the first branchiostegal ray, with a central rod of bone and cartilage, and are controlled by modified muscles of the hyoid system. These are features which promote sufficient rigidity and strength to enable their use as excavating devices in the surface sediments of the reef. In an electrophysiological study of foraging in the goatfish Parupeneus porphyreus, Holland (1976) found that the removal of barbels adversely altered and greatly reduced arousal behaviour, and eliminated food-search behaviour. When the sense of smell was effectively removed by cauterizing the nasal rosettes of the olfactory apparatus, normal arousal and food-search behaviour was recorded. This stresses the important role of the barbels in the acquisition of prey, and that it is crucial for the barbels to be equipped for a fully functional role in the detection and manipulation of prey items as soon as the fish metamorphoses and settles.

Barbels sometimes do not separate from the branchiostegal arch until late in the pelagic life, commonly at 16 - 18 mm SL. In one fish, barbels were still found to be attached by a membrane connection at 22 mm SL, which represents approximately three-quarters of its pelagic existence. This suggests that wholly functional barbels are not used actively in the pelagic phase; a conclusion which is supported by tank observations of newly caught pelagic individuals.

Other researchers have found large changes in the sensory systems of marine species during metamorphosis. Most examples come from the flatfish where metamorphosis proceeds through a series of well defined stages, involving the proliferation of functioning larval structures and regression of tissues (Youson 1988). Examples are the migration of the eye in flounder (e.g. Ryland 1966), the creation of the asymmetry in the projections of the right and left olfactory bulbs in Pseudopleuronectes americanus (Rao and Finger 1984), or the development of the lateral line system in Pleuronectes platessa and Scophthalamus maximus (Neave 1986). None have found as rapid a change as that presented for the reef-fish in this study (e.g. Neave 1984). Obviously, further studies of the sensory

systems of reef fishes are required before the generality of the magnitude and nature of these changes can be assessed.

This study has shown that major changes to the sensory system can occur at settlement in reef fishes. Youson (1988) noted that if a major remodelling of the body systems takes place after a relatively uneventful larval period, then the timing of the initiation of metamorphosis is undoubtedly of great significance to the ultimate survival of the post-metamorphic individual. To achieve some understanding of the dynamics of coral reef fish populations it is important to examine how sensory competence and the physiological status of the organism (metamorphosis often involves the mobilisation of storage reserves) interrelates with the actual timing and location of settlement onto the reef. Furthermore, since reef fish may actively choose when and where they settle (e.g. the damselfish, Dascyllus aruanus, Sweatman 1985b), it will be of interest to know to what extent sensory development governs settlement, and to what extent a fish can delay metamorphosis (e.g. Victor 1986) once it has reached a level of development at which the process can occur.

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Figure Legends

Fig. 1. General morphology of the regions of the eye and barbel discussed. A. Cross-section through the eye of a pelagic Upeneus tragula, showing a stylised representation of the layers of the retina (note the double-cone layer). B. Cross-section through the sensory barbel, showing details of the cell layers examined.

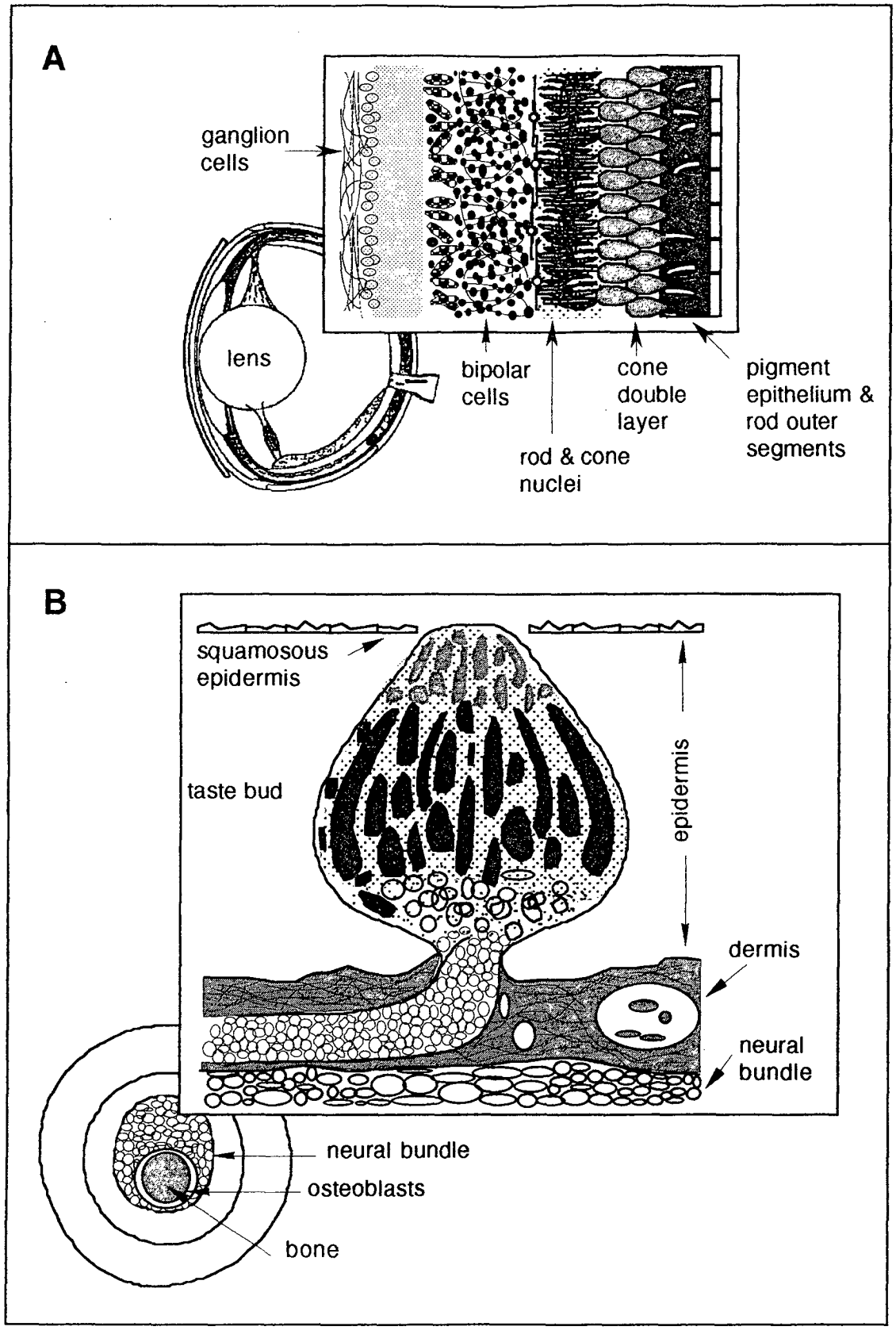
Fig. 2. Change in the density of cones (per 0.01 mm^2) in the dorsal retina of Upeneus tragula with fish standard length (mm) (n = 10).

Fig. 3. Change in the number of photoreceptors (cones and rods) converging onto the bipolar (processing) cells during growth of Upeneus tragula. Symbols as for Fig. 2.

Fig. 4. Change in the length of the sensory barbels (mm) with standard length of the fish. Symbols as for Fig. 2.

Fig. 5. Change in the cross sectional area of the barbel (x 10^3 mm^2) with standard length of fish. Symbols as for Fig. 2.

Fig. 6. Change in the percentage contribution of epidermal tissue to the total cross-sectional area of the barbel with length of fish. Symbols as for Fig. 2.



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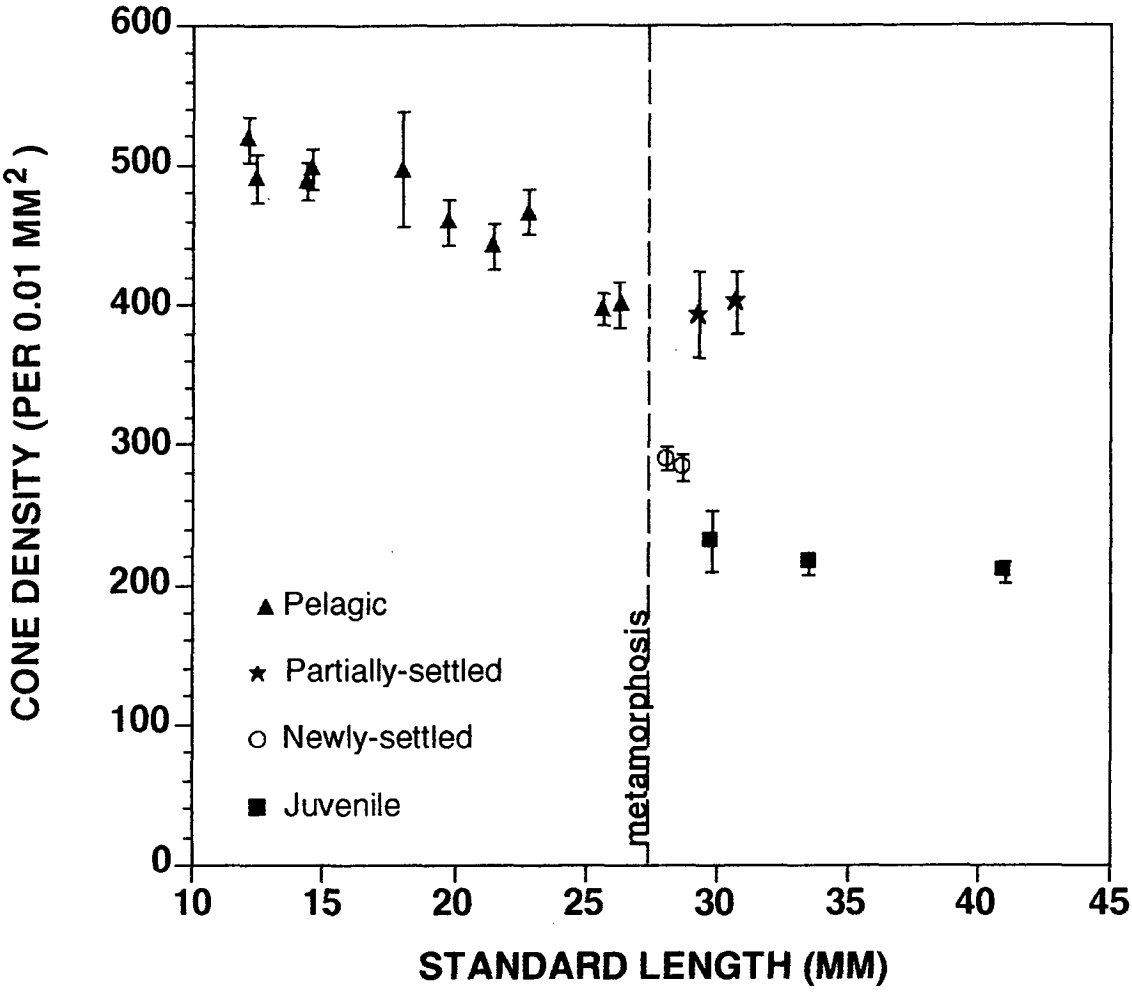

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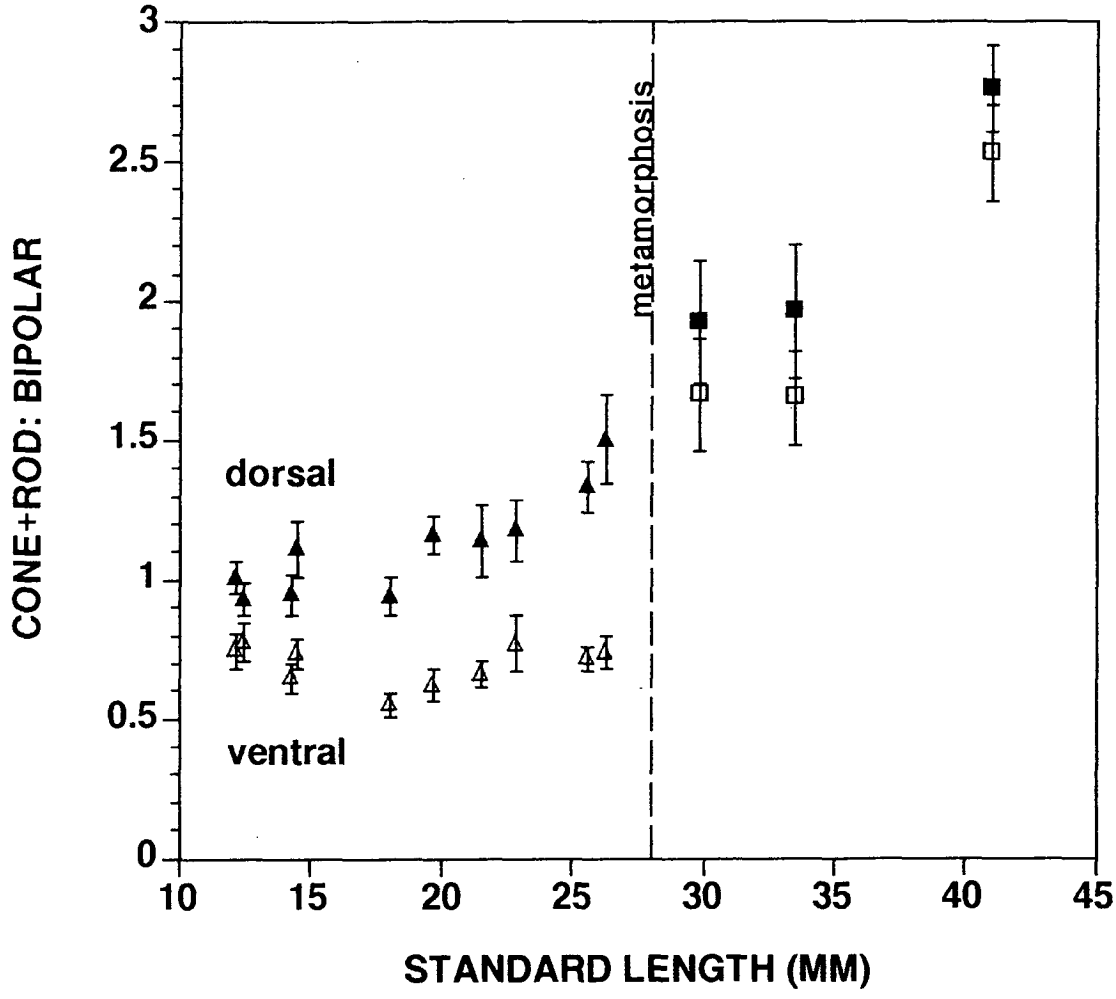

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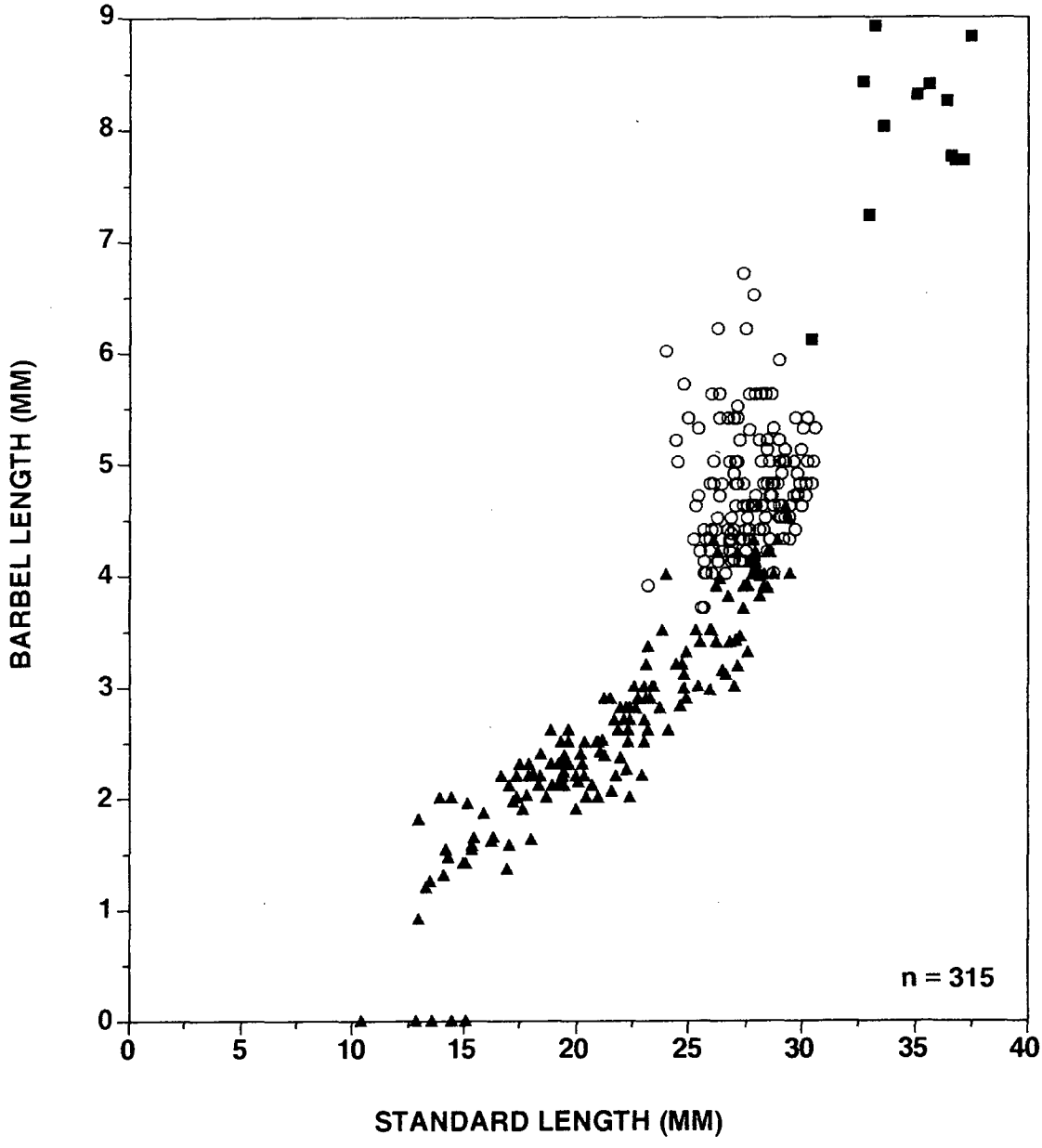

Figure 4. Change in the length of the sensory barbels (mm) with standard length of fish. Symbols as for Fig. 2.

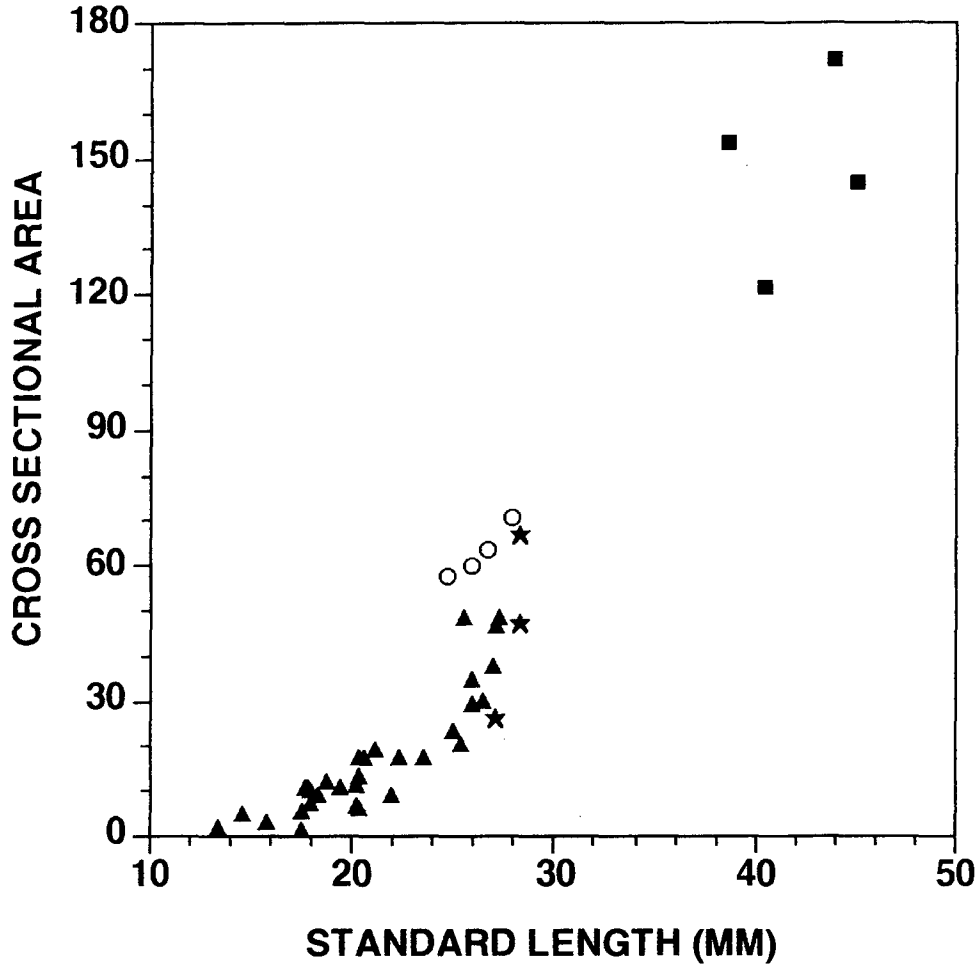

Figure 5. Change in the cross-sectional area of the barbel (x $10^3 \mu m^2$) with standard length of fish. Symbols as for Fig. 2.

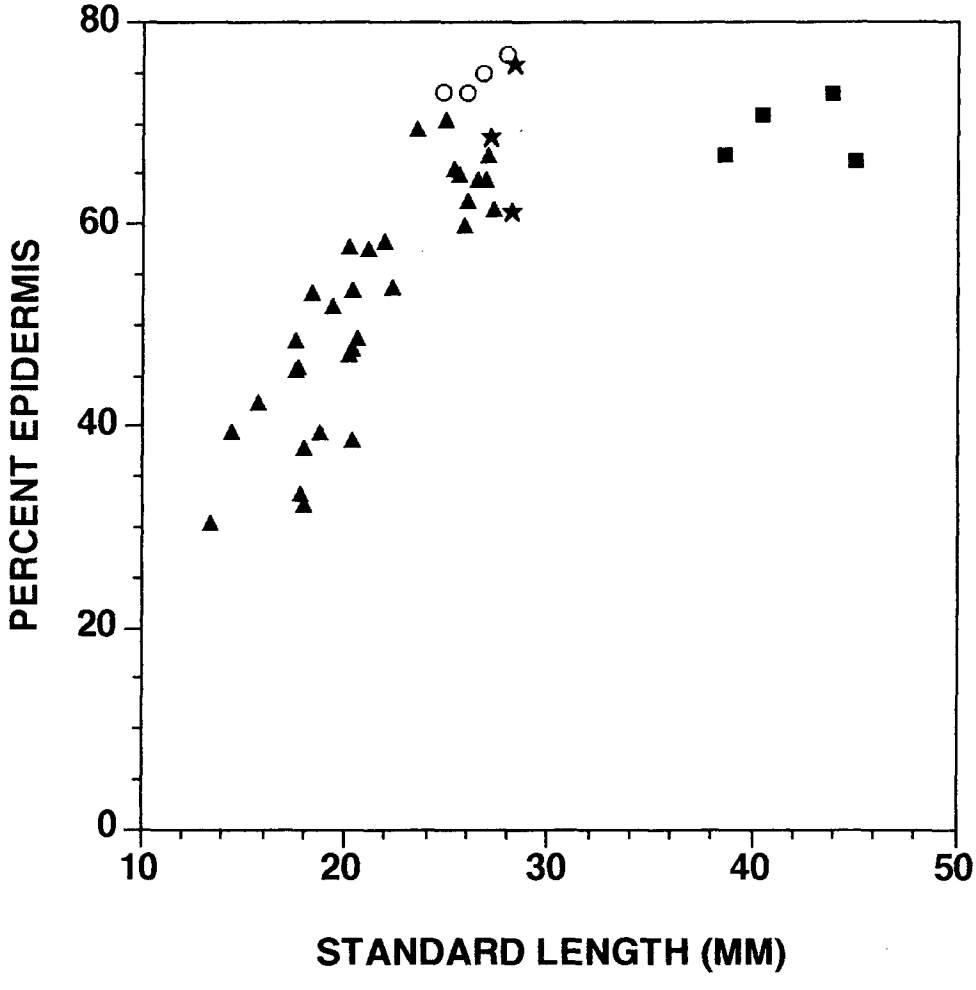

Figure 6. Change in the percentage contribution of epidermal tissue to the total cross-sectional area of the barbel with length of fish. Symbols as for Fig. 2.

Appendix 2

Predation experiments

A2.1 INTRODUCTION

Newly settled *Upeneus tragula* have high levels of variability in body condition (Chapter 4, 5). It is important to know whether this variability extends through to the juvenile population, or whether it is altered by the initially high levels of predation on naive settlers (reviewed by Hixon 1991). This study experimentally examines whether body size or body condition of newly settled fish influences the probability of them being preyed upon.

A2.2 MATERIALS AND METHODS

Experiment 1: *Is the size distribution of newly settled reef fish influenced by predation ?*

Pelagic stage *Upeneus tragula* were collected using a plankton mesh purse-seine deployed around floating aggregation rafts (see Chapter 2 for details). All fish caught were transferred with a dip net from the purse seine into black plastic tanks of aerated seawater. These were brought back to the laboratory, where overnight some fish underwent metamorphosis and settled. Fifty newly settled *Upeneus tragula* of the broadest possible size range were introduced into a tank (81 x 35 x 36 cm) with sand and seagrass. Seagrass was organised into two square patches separated by a narrow corridor and took up one-third of the area of the bottom of the tank. The standard length of the fish was measured with calipers during their transferral into the experimental tank, by placing each fish in a small clip-seal plastic bag partially filled with water. This allowed direct restraint of the fish without scale damage. A comparison of the standard length measurements from live fish using this method with measurements made after the fish had been killed by cold shock found that the latter were precise (mean 0.4% underestimate, standard error 0.27, n = 10). Fish were left 12 to 18 hours to acclimate, and then two predatory lizardfish (*Synodus variegatus*) were introduced (~120 mm SL) within six hours of being caught. *S. variegatus* occurs in the same habitats as *U. tragula* and is a common predator of this goatfish (pers. obs.). *U. tragula* were fed 36 to 48 h old

Artemia sp. nauplii (Artemia 90 brand) throughout the experiment. The lizardfish were removed from the tanks when approximately half of the initial fish remained. *U. tragula* were then killed and re-measured.

A control for growth during the experiment was run concurrently. The tank set-up, measurement of the fish and feeding regime was similar, but no lizardfish were added to the tank. Fish were re-measured when the associated predator trial was terminated. The mean increase in standard length over the experiment for the control fish was subtracted from the lengths of fish that remained after the predation trial had been terminated. This accounted for fish growth over the experimental period and allowed comparisons of the size distributions before and after the predation episode. The experiment was repeated three times. Trials were pooled to test for a difference between size frequency distributions of *Upeneus tragula* before and after predation.

Experiment 2: *Does prey condition influence its ability to escape predators ?*

Pelagic stage *U. tragula* were collected (as above) and fish of 20 - 23 mm SL were placed randomly into six 60 l tanks at densities of about 50 per tank. Tanks were assigned to one of two feeding regimes: fed *ad libitum* (1600 nauplii/l); fed 2/5 *ad libitum* (640 nauplii / l). Food was added to each of the tanks four times a day, in concentrations that were calculated to result in the noted densities of *Artemia* sp. after introduction. To quantify the initial condition of the newly settled fish at the start of the experiments, 10 newly settled fish were removed from each feeding treatment for biochemical analysis of their body constituents. The two treatments were found to significantly differ in lipid content (Means: fed *ad libitum*, 37.2 mg/g wet wt; 2/5 fed, 18.7 mg/g wet wt; $F_{1,18} = 42.12$ $p < 0.0001$).

Three trials were established one week apart, as sufficient individuals settled in the tanks and could be used for the experiment. When 20 fish had settled over all 3 tanks within a treatment, they were pooled into a single trial. In two of the predation trials, newly settled *U. tragula* from the fed *ad libitum* treatment were marked by immersion for 12 hours in aerated tetracycline-hydrochloride (250 mg/l). In the remaining trial, fish from the 2/5 fed treatment were marked with tetracycline. This prevented confounding of the experiment by the potential influence of tetracycline on the susceptibility of the fishes to

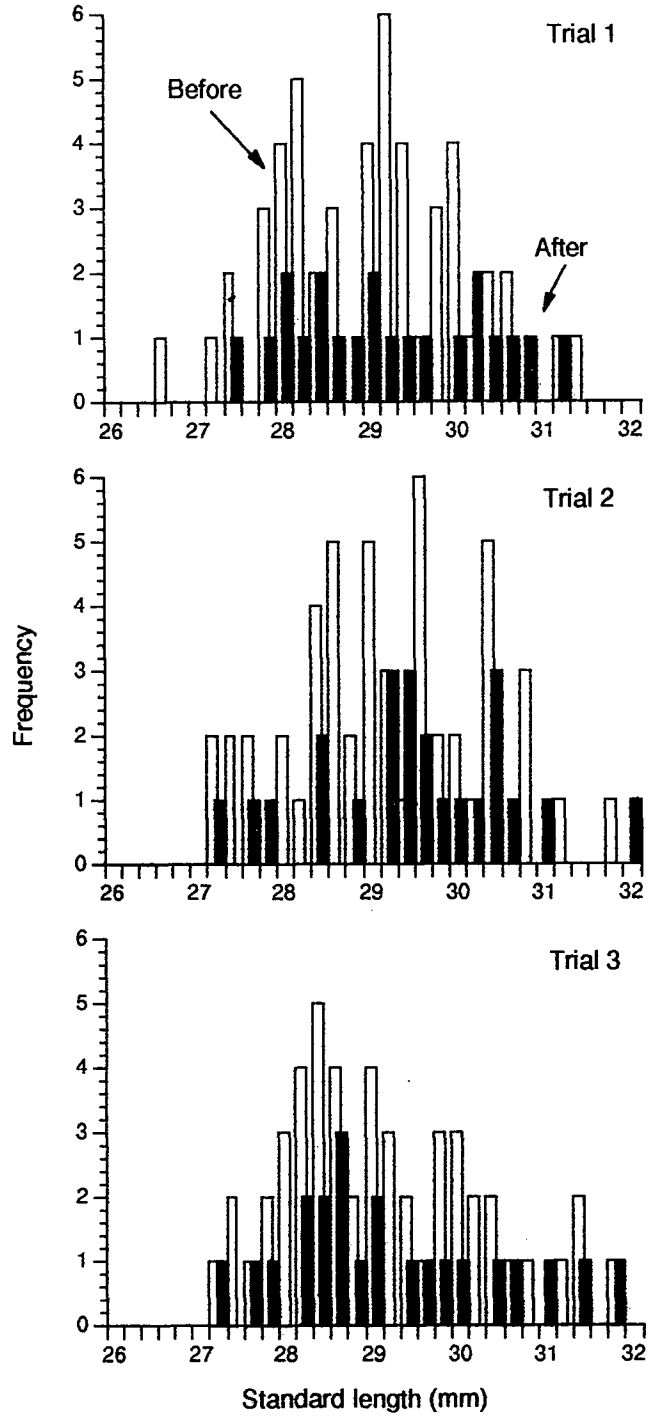

Figure A2.1. The results of three independent trials of the effects of predation by two lizardfish, *Synodus variegatus*, on the size distribution of newly settled *Upeneus tragula*. Size distributions of *U. tragula* before predation (plain bars) are compared to the size distributions after approximately half the fish had been eaten (solid bars). A homogeneity test between size distributions before and after predation, pooled over the 3 trials, was non-significant ($\chi^2_{11df} = 3.962, p > 0.95$).

predation. Twenty fish from each treatment were placed into a tank with sand and seagrass patches. Fish were given 12 - 18 hours to acclimate before the introduction of two lizardfish predators. The experiment was concluded when half of the fish had been eaten. Remaining fish were killed by cold shock, weighed and measured. Their otoliths were removed and examined under U/V light to detect the fluorescence characteristic of a fish treated with tetracycline. In this way fish could be assigned to either of the two initial feeding treatments.

A2.3 RESULTS AND DISCUSSION

In three trials of the first experiment, the size distribution of the prey was not altered by predation (Fig. A2.1). In only one of the three trials were the largest or smallest individuals removed by predation. This suggests that predation by lizardfish does not reduce the size range of the prey species and that predation was random with respect to prey size.

The second experiment, which examined whether body condition of the prey species influenced its probability of survival, obtained a similar result. In three trials the proportion of the surviving fish that had started their settled life in high biochemical condition (fed *ad libitum* treatment) was 50%, 47.4%, 52.2%. This suggests that susceptibility of the prey to predation is not influenced by their biochemical condition.

Lizardfish in a seagrass habitat are ambush predators that either bury themselves in the sand or wait motionless on a piece of rubble, camouflaged by their disruptive colouration. Therefore, it is not so surprising that predation by lizardfish had no effect on the range of size or condition of *Upeneus tragula*, since fish taken are those that by chance stray too close. However, strikes at prey were not always successful, and if a lizardfish missed its first target it would rapidly strike out at the next closest fish before settling back into the sand. A similar size-independent result would be obtained if the probability of prey survival was directly related to their burst speed. Chapter 5 showed that burst speed of newly settled *Upeneus tragula* was not related to fish size or condition. It is not until similar experiments have been conducted with other cryptic predators, and overt predators such as the coral trout *Plectropomas leopardus*, that the importance of the variability in the quality of fish at settlement can be confidently assessed.